

Yield-trait and SSR-based genetic diversity and population structure analysis in Northeastern Himalayan germplasm collection of Job's tears (*Coix lacryma-jobi* L.)

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Abstract

The study characterized 34 Job's tears accessions from the Northeastern Himalayan region using yield-related traits and SSR markers. Genotyping with 17 SSR markers revealed an average of 3.18 alleles per locus, varying from 2 to 4. PIC values ranged from 0.27 to 0.52, averaging 0.41. Clustering and PCoA based on SSR markers grouped the accessions into three major groups. The first three principal coordinates in the PCoA cumulatively explained 41.96% of the variability present in the accessions, with the first and second principal coordinates explaining 22.51% and 10.39% of the molecular variability, respectively. The model-based genetic structure analysis detected the maximal ΔK (80.87) at $K = 2$, grouping the entire accessions into two subgroups. AMOVA indicated higher genetic variation within individuals (52.86%) than among individuals (47.14%). The high F_{ST} value (0.2) suggested significant differentiation between populations. Yield-trait-based analysis resulted in four clusters. Cluster I included six accessions with short height, Cluster II consisted of 12 accessions with high test weight, Cluster III comprised six accessions with high yield, and Cluster IV had nine accessions with early flowering, respectively. PCA extracted three components explaining 67.4% of total variation, with PC1 emphasizing yield and growth-related traits and PC2 highlighting days to flowering and panicle length. Most traits showed positive correlations with yield per plant, especially test weight (0.65, $P \leq 0.01$). Path coefficient analysis revealed a strong positive direct effect of test weight and the number of internodes per plant on yield per plant. Cluster analysis and GT biplot analysis showed similar patterns among accessions. However, the Mantel test indicated no significant correlation between genetic and morphological distances ($r^2 = 0.010$, $P = 0.41$). AMMI analysis and MTSI for yield-related traits identified three promising accessions (IC600638, IC540181, and IC540256) suitable for selection at 10% intensity in future breeding programs.

Introduction

Job's tears (*Coix lacryma-jobi* L.) ($2n = 4x = 20$; genome size ~ 1.7 Gb) is an underutilized cereal crop of the tribe Andropogoneae and subfamily Panicoideae in the family Poaceae (Kang et al. 2020). It exists either as annual or perennial, and can grow up to 3 meters in height. They possess solid internodes and cauline leaves with finely fringed margins arranged distichously. The axillary inflorescences consist of the pistillate and the staminate racemes. The pistillate racemes are enclosed within a modified leaf sheath called involucre, which is globose-ovoid, bony or soft, and typically measures 8–12 mm in length, displaying various colors such as white, greyish, bluish, or black. The staminate racemes are flexible and extend on a long peduncle from the mouth of the involucre. The involucre encloses the ellipsoid to subglobose, ventrally furrowed caryopsis, measuring 2.5–5 mm in length (Thieret 2003). Both self and cross-pollination occur in Job's tears, with the latter being predominant (Jansen 2006). There are four varieties of Job's tears, namely, *C. lacryma-jobi* variety (var.) *lacryma-jobi*, *ma-yuen*, *puellarum*, and *stenocarpa*. *C. lacryma-jobi* var. *lacryma-jobi* is a common wild variety bearing seeds with a thick, stony hull, while *C. lacryma-jobi* var. *ma-yuen* is a cultivated variety with a thin, papery hull. The stony hull of the wild variety provides protection against adverse environmental conditions, while the papery hull highlights the fundamental trait for domesticating Job's tears (Devaraj et al. 2020). Job's tears possess a massive root system with fibrous roots and are resistant to drought and waterlogging.

Job's tears is native to Northern Indo-China and the Eastern Himalayan region (Arora 1977; Areces-Berazain et al. 2017; Diao 2017; Huang et al. 2019). It has recently been introduced as a cereal, fodder, and forage crop to most tropical and subtropical regions of the world (Shen et al. 2019). Job's tears is often called "Green Food" and is used in various preparations, including powder, biscuits, and several alcoholic and non-alcoholic beverages (Shen 2017). Its seeds contain high-quality carbohydrates, fatty acids, protein and amino acids, vitamins, and minerals. Seed fat ranges from 6.34–7.70%, protein 12.88–15.95%, and starch 58.34–63.80% (Tan et al. 2017). Specifically, among cereal crops, Job's tears has the highest seed protein content. Besides being used as a nutriceal, Job's tears seeds have been used in Chinese folk medicine since antiquity. It is reported to have anticancer, hypolipidemic, hypoglycemic, antioxidant, anti-inflammatory, and anti-allergic properties (Yeh et al. 2006; Chen et al. 2011; Chung et al. 2011; Chen et al. 2012; Wang et al. 2012). An anticancer drug derived from the seed oil of Job's tears, known as Kanglaite, has been utilized in clinical applications in China and demonstrated efficacy in the treatment of various types of cancer (Li 2012). Job's tears plant produces an array of secondary metabolites, such as benzoxazinoids, lactams, and phytosterols (Choi et al. 2019). Coixol, a benzoxazinoid, is of particular interest because it is found only in Job's tears (Zhu 2017) and exhibits a variety of physiological activities, including pharmacological effects (Choi et al. 2017). Owing to its high nutritional value and pharmacological effects, the demand for Job's tears is increasing rapidly worldwide. Furthermore, Job's tears is gaining recognition within the scientific community as a promising genetic resource for comparative genomic studies aimed at enhancing other cereal crops. This is attributed to its notable nutritional values and resilience to a range of biotic and abiotic stresses, including disease resistance, cold tolerance, low pH tolerance, resistance to waterlogging, and the development of robust root systems (Liu et al. 2020).

Most studies on Job's tears, so far, have focused mainly on its agronomic characteristics (Liang et al. 2006; Wang et al. 2013; Li et al. 2015), chemical components (Huang et al. 2009; Chen et al. 2011), and pharmacological functions (Kuo et al. 2001; Huang et al. 2005; Huang et al. 2009; Sato and Osawa 2010; Wang et al. 2012), and there are only a few reports on the genetic variation and relationships of Job's tears germplasm (Li et al. 2001; Ma et al. 2010; Wang et al. 2015). To the best of our knowledge, there is hardly any report from India. Exploration and germplasm collection initiatives have also been rare in India. Consequently, only a limited Job's tears germplasm is available in the National Gene Bank of India housed at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi. In this study, we analyzed 34 germplasm accessions collected from the Northeastern Himalayan states of India, including Nagaland, Arunachal Pradesh, Meghalaya, Mizoram, and Assam using yield and yield-related traits and SSR markers to assess the extent of diversity and identify promising genotype(s) for future breeding programs.

Material and methods

Plant material and experimental design

We used 34 Job's tears accessions collected across the Northeastern Himalayan states of India, including Nagaland (13), Arunachal Pradesh (10), Meghalaya (2), Mizoram (1), and Assam (1). The site of collection of seven germplasm accessions remains unidentified (Supplementary Table 1). The germplasm accessions were evaluated for yield and yield-related traits for three consecutive years (2018–20) in a randomized complete block design (RCBD) with three

replications at the experimental farm of the ICAR Research Complex for NEH Region, Umiam, Meghalaya, India. Sowing was done in June in 5 rows of 4 m row length with a 60 cm row-to-row spacing. Manual weeding and earthing were done 35–40 days after sowing. The crop was harvested at 80% maturity during November after drying of leaves.

Data recording on yield and yield-related traits

We recorded the data for nine yield and yield-related traits, namely, days to 50% flowering, days to 80% maturity, plant height (cm), number of tillers per plant, panicle length (cm), leaf length (cm), number of internodes per plant, test weight (g) and yield per plant (g) from five randomly chosen plants. The mean value of all five plants constituted one replication.

SSR-based genotyping

We extracted high molecular weight genomic DNA from 20-day-old Job's tears seedling leaf tissue using the CTAB method (Murray and Thompson 1980). After washing the DNA pellet with 70% ethanol and air-drying, we dissolved it in sterile TE buffer and stored it at 4°C. To eliminate RNA contamination, we treated the DNA with RNase A and analyzed its quality on a 1.0% agarose gel, quantifying it with a NanoDrop™ OneC microvolume UV-Vis spectrophotometer. Following quantification, we diluted the DNA to 20 ng/μL for PCR reactions. Genetic diversity among Job's tears accessions was assessed using 17 SSR primers. PCR reactions were carried out with 40 ng of template DNA, 0.2 mM of each dNTP, 0.5 U of Taq DNA polymerase, and 0.2 mM of each forward and reverse primer in a 20 μL reaction mixture. The amplifications were conducted in an Eppendorf Mastercycler Gradient PCR machine, and the SSR products were resolved on a 3.5% agarose gel with ethidium bromide. Gel images were captured using a Gel Doc™XR + Imaging System, and amplicon sizes were determined against a 50bp DNA ladder.

Data analysis

The mean of the data collected from randomly chosen five plants of each genotype, constituting one replication, was used for the analysis. Analysis of variance (ANOVA) for yield and yield-related traits, estimation of genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), broad-sense heritability (H^2_b), genetic advance as a percentage of mean (GAM), correlation, and path analysis were executed in R Studio v.4.2.1 (<https://cloud.r-project.org/package=variability>) (Popat et al. 2020) using the R script. Clustering was performed using the Stats package in R v.4.2.1 (R Core Team 2012) and visualized with the Factoextra package (Kassambara and Mundt 2020). Principal Component Analysis (PCA) was conducted using the Paleontological Statistics Software Package (PAST v.4.0) (Hammer et al. 2001). The multi-trait stability index (MTSI), selection differential, and response to selection were calculated using functions in the 'metan' package (Olivoto and Lucio 2020) in R v.4.2.1.

We computed the statistics for SSR-based analysis, including the effective number of alleles (EN), observed heterozygosity (Ho), expected heterozygosity (He), Shannon's Information Index (I), and the Polymorphic Information Content (PIC) across genotypes. We used Nei's genetic distance to perform genetic-distance-based clustering with PowerMarker v.3.25 (Liu and Muse 2005) and POPGENE v.1.32 software (Yeh et al. 1999). To infer population structure among genotypes, the model-based clustering program STRUCTURE v.2.3.4 (Pritchard et al. 2000) with the admixture model was used. The number of subpopulations (K) was set from 1 to 10, with a burn-in period of 50,000 and a run length of 50,000 Markov Chain Monte Carlo numbers (MCMC) and 10 individual replications for each K value. The determination of the number of subpopulations was performed using both LnP (D) and Evanno's DK methods (Evanno et al. 2005) through the web-based program STRUCTURE HARVESTER (Earl and vonHoldt 2012). Principal Coordinate Analysis (PCoA) to understand relationships among the genotypes and the analysis of molecular variance (AMOVA) were conducted using GenAlEx v.6.5 (Peakall and Smouse 2012). Wright's F statistics were calculated using Arlequin v.3.5.2.2. Mantel test, calculating the correlation between morphological and genetic distance, was performed using GenAlEx v.6.5.

Results

Genetic variability for yield and yield-related traits

All nine yield and yield-related traits, including days to 50% flowering, days to 80% maturity, plant height (cm), number of tillers per plant, panicle length (cm), leaf length (cm), number of internodes per plant, test weight (g), and yield per plant (g), exhibited highly significant variation ($P \leq 0.01$) among all 34 Job's tears accessions. The GCVs and PCVs ranged from 0.56% for days to 80% maturity to 36.98% for yield per plant and 0.74% for days to 80% maturity to 49.37% for yield per plant, respectively. The GCV values for plant height, number of tillers per plant, panicle length, and number of internodes per plant were moderate ($> 10\%$), while these values for leaf length, test weight, and yield per plant were high ($> 20\%$). Conversely, the PCV values indicated that only plant height and panicle length had moderate values ($> 10\%$), whereas the number of tillers per plant, leaf length, number of internodes per plant, test weight, and yield per plant exhibited high PCV ($> 20\%$). In general, the PCVs were higher for all the traits when compared to the GCVs. Days to 50% flowering (96.0%), plant height (74.0%), leaf length (61.0%), and test weight (99.0%) exhibited high heritability whereas, days to 80% maturity (58%), panicle length (34%), number of internodes per plant (60%), and yield per plant (56%) showed medium to low heritability; number of tillers per plant being the lowest (8%). Genetic advance over mean ranged from 0.88 for days to 80% maturity to 61.21% for test weight. Test weight (61.21), yield per plant (57.07), leaf length (32.79), and number of internodes per plant (28.74) exhibited a high genetic advance over the mean. The details of the genetic parameters for yield and yield-related traits are indicated in Table 1.

Table 1
Genetic parameters for yield and yield-related traits in Job's tear

Parameter	DOF	DOM	PH	NT	PL	LL	Nls	TW	YPP
Mean	84.45	157.96	206.03	4.40	45.04	49.31	6.92	11.98	59.28
Max	93.00	162.00	302.00	12.00	82.00	76.00	10.00	26.10	217.00
Min	75.00	153.00	91.00	1.00	13.00	16.00	4.00	4.60	2.61
Sem	0.39	0.44	7.70	1.01	2.49	3.62	0.56	0.17	3.42
CD (5%)	1.09	1.24	21.73	2.85	7.03	10.22	1.57	0.49	9.66
GV	12.16	0.80	509.41	0.27	9.63	61.00	1.41	14.72	44.92
PV	12.61	1.38	687.21	3.33	28.22	100.29	2.33	14.81	80.07
GCV	4.18	0.56	13.80	11.97	10.68	20.41	17.96	29.81	36.98
PCV	4.25	0.74	16.03	42.38	18.29	26.17	23.12	29.90	49.37
h ₂ b	0.96	0.58	0.74	0.08	0.34	0.61	0.60	0.99	0.56
GAM	8.45	0.88	24.48	6.96	12.85	32.79	28.74	61.21	57.07

Relationship among the yield and yield-related traits

Pearson's correlation study conducted to illustrate the relationship between the yield and yield-related traits (Table 2) revealed a significant positive correlation of yield per plant with plant height (0.55), number of tillers per plant (0.44), leaf length (0.43), number of internodes per plant (0.57), and test weight (0.64). Test weight exhibited a highly significant ($P \leq 0.01$) and positive correlation with plant height (0.56), number of tillers per plant (0.43), leaf length (0.47), and number of internodes per plant (0.43). Likewise, the number of tillers per plant showed a significant correlation with plant height (0.37). On the contrary, days to 50% flowering exhibited a significant negative correlation with plant height (-0.35) and the number of tillers per plant (-0.46), and days to 80% maturity showed a significant negative correlation with leaf length (-0.42).

Table 2
Pearson's correlation among yield and yield-related traits in Job's tear

	DOF	DOM	PH	NT	PL	LL	Nls	TW	YPP
DOF	1	0.11	-0.35*	-0.46**	0.10	-0.09	0.02	-0.19	-0.17
DOM		1	-0.31	-0.21	-0.12	-0.42*	-0.1	-0.21	-0.34
PH			1	0.37*	0.21	0.34*	0.54**	0.56**	0.55**
NT				1	0.15	0.33	0.47**	0.43**	0.44**
PL					1	0.47**	0.16	0.10	0.22
LL						1	0.24	0.47**	0.43*
Nls							1	0.43*	0.57**
TW								1	0.64**
YPP									1

Multivariate analysis based on yield and yield-related traits

Clustering based on the yield and yield-related traits grouped the Job's tears accessions into four major clusters, cluster II being the largest with 12 accessions and clusters I and III being the smallest each with six accessions (Fig. 1). One accession (IC374506) did not show affiliation to any of the clusters and served as an out-group. Cluster I grouped all the short-height accessions (≤ 183.17 cm), while cluster II grouped the accessions with higher test weights (≥ 13.99 g). Cluster III consisted of accessions with high yields (≥ 71.72 g/plant). A total of nine accessions showed their affiliation to cluster IV, all of which were of early flowering type (≤ 83.29 days) (Table 3). The first three principal components accounted for 67.47% of total variation for all yield and yield-related traits (Table 4) and exhibited high correlation among the characteristics analyzed. PC I accounted for 40.75% of the total variation and was dominated by the traits like plant height, number of tillers per plant, leaf length, number of internodes per plant, test weight, and yield per plant. PC-II accounted for 14.66% of the total variation and the most influential traits were days to 50% flowering, panicle length, and leaf length. The PC-III accounted for 12.06% of the total variation with days to 50% flowering and days to 80% maturity being the predominant traits.

Table 3
Comparison of clusters based on yield and yield-related traits

Cluster	Parameter	DOF	DOM	PH	NT	PL	LL	Nls	TW	YPP
I	Mean	87.03	158.28	183.17	3.32	46.25	48.30	6.18	9.05	38.18
	Max	90.00	158.70	199.80	3.70	49.40	53.10	6.60	11.10	58.40
	Min	83.90	157.90	160.10	2.90	41.40	42.30	5.90	6.60	21.20
II	Mean	83.86	157.90	215.08	5.00	45.33	51.96	7.57	13.99	68.85
	Max	85.90	158.70	234.90	5.80	49.40	56.10	8.10	19.20	84.70
	Min	80.90	156.90	193.70	4.10	40.80	47.30	6.90	10.10	54.40
III	Mean	84.60	156.98	216.83	4.37	47.28	49.98	6.90	12.72	71.72
	Max	86.10	157.40	222.30	5.10	53.20	54.60	7.40	14.60	90.10
	Min	83.10	156.70	203.60	3.40	42.80	41.40	6.60	10.50	59.20
IV	Mean	83.29	158.41	205.66	4.36	43.33	47.51	6.51	11.10	50.16
	Max	86.30	159.10	221.20	5.10	47.90	54.10	7.30	13.30	59.30
	Min	80.20	157.40	183.30	3.90	37.40	42.80	5.80	8.90	34.60
Out-group	Mean	85.70	159.00	175.30	3.70	35.30	36.10	7.40	8.10	45.60

Table 4
PCA for yield and yield-related traits in Job's tear

	PC-I	PC-II	PC-III
DOF	-0.18	0.60	0.48
DOM	-0.25	-0.18	0.51
PH	0.40	-0.13	0.08
NT	0.36	-0.29	-0.10
PL	0.19	0.59	-0.12
LL	0.34	0.40	-0.31
Nls	0.35	-0.02	0.57
TW	0.40	-0.07	0.16
YPP	0.43	0.02	0.19
Eigen value	3.67	1.32	1.09
% variance	40.75	14.66	12.06
Cumulative variance	40.75	55.41	67.47

The PCA mostly confirmed the cluster analysis. The accessions grouped together in cluster analysis were present nearer in the genotype-by-trait (GT) biplot (Fig. 2). In this biplot, an acute angle between two traits indicates a positive correlation, whereas an obtuse angle signifies a negative correlation (Yan and Fregeau-Reid 2018). Acute angles were observed between plant height, number of tillers per plant, number of internodes per plant, test weight, and yield per plant, indicating positive correlation between them.

Regression studies using path analysis

The direct and indirect effects of various yield and yield-related traits on yield per plant were computed using phenotypic correlations (Table 5). Number of internodes per plant (0.35) and test weight (0.41) exhibited a very high positive direct effects, whereas days to 50% flowering (0.08), days to 80% maturity (0.19), number of tillers per plant (0.11), and panicle length (0.10) exhibited moderate direct effects on yield per plant. Plant height (0.23), number of tillers per plant (0.18), leaf length (0.19), and number of internodes per plant (0.18) exhibited high positive indirect effects on yield per plant *via* test weight. Likewise, plant height (0.19), number of tillers per plant (0.16), and test weight (0.15) also exhibited high positive indirect effects on yield per plant *via* the number of internodes per plant. The number of internodes per plant and test weights were the major determining traits for the yield per plant. A low residual effect (0.32) revealed that the yield and yield-related traits considered for the study were appropriately selected.

Table 5
Path analysis of yield and yield-related traits in Job's tear

	DOF	DOM	PH	NT	PL	LL	Nls	TW
DOF	0.08	-0.02	-0.01	0.00	0.01	0.00	0.01	-0.08
DOM	-0.01	0.19	-0.01	0.00	-0.01	-0.01	-0.04	-0.09
PH	0.03	0.06	0.03	0.00	0.02	0.01	0.19	0.23
NT	0.04	0.04	0.01	0.11	0.01	0.01	0.16	0.18
PL	-0.01	0.02	0.01	0.00	0.10	0.01	0.06	0.04
LL	0.01	0.08	0.01	0.00	0.05	0.02	0.08	0.19
Nls	0.00	0.02	0.01	0.00	0.02	0.00	0.35	0.18
TW	0.02	0.04	0.01	0.00	0.01	0.01	0.15	0.41
Residual Effect = 0.32								

SSR-based genetic diversity analysis

Seventeen SSR markers were used for the genetic diversity analysis of Job's tears accessions. Table 6 presents the summary of genetic diversity statistics for all 17 SSR loci. A total of 54 alleles were detected, ranging from two (GBssrJT31 and GBssrJT32) to four (GBssrJT68, GBssrJT136, GBssrJT174, and GBssrJT181), with an average of 3.18 alleles per locus. The effective number of alleles (Kimura and Crow, 1964) ranged from 1.22 (GBssrJT157) to 2.73 (GBssrJT198), with an average value of 2.12. Shannon's Information Index was highest (1.04) for GBssrJT149. The PIC values ranged from 0.27 (GBssrJT157) – 0.52 (GBssrJT130) with an average of 0.41.

Table 6
Summary of SSR markers used for the analysis of Job's tear germplasm

Locus	Repeat motif	NA	EN	Ho	He	I	PIC
GBssrJT25	GCA)4CCA(GCA)2, (TGG)5	3.00	1.84	0.27	0.46	0.79	0.39
GBssrJT31	(GCC)5, (CCG)4	2.00	1.34	0.42	0.25	0.44	0.28
GBssrJT32	(TGGCTGC)4	2.00	1.57	0.45	0.32	0.54	0.31
GBssrJT41	(CTT)3TTC(CTT)3TTC(CTT)2	3.00	2.13	0.43	0.51	0.83	0.51
GBssrJT68	(CTCCTG)2(CTC)4	4.00	1.99	0.28	0.49	0.89	0.46
GBssrJT130	(GGC)6	3.00	2.41	0.65	0.58	0.96	0.52
GBssrJT136	(CATG)3, (CGA)4, (GAG)4	4.00	2.57	0.13	0.43	0.87	0.48
GBssrJT149	(TTCAT)4	3.00	2.47	0.21	0.59	1.04	0.59
GBssrJT157	(GCT)4	3.00	1.22	0.02	0.02	0.06	0.27
GBssrJT161	(CAT)6	3.00	2.37	0.27	0.56	0.89	0.38
GBssrJT164	(CCTCCG)2	3.00	1.58	0.29	0.33	0.57	0.29
GBssrJT170	(CTG)1T(CTG)2CTC(CTG)1T(CTG)4	3.00	2.58	0.11	0.36	0.76	0.42
GBssrJT174	(GAGGA)3	4.00	2.15	0.51	0.54	0.87	0.45
GBssrJT181	(AG)13	4.00	2.29	0.18	0.49	0.92	0.47
GBssrJT183	(CGC)4	3.00	2.50	0.14	0.51	0.94	0.41
GBssrJT185	(GGC)5	3.00	2.31	0.19	0.55	0.92	0.40
GBssrJT198	(GAT)4GAGGA(GGAC)3	4.00	2.73	0.16	0.48	0.95	0.32
NA = No. of alleles; EN = Effective no. of alleles; Ho = Observed heterozygosity; He = Expected heterozygosity; I = Shannon's Information Index; PIC = Polymorphism Information Content							

Genetic structure and interrelationship

The Nei's genetic distance-based clustering grouped the 34 Job's tears accessions into three major clusters (Fig. 3). Cluster II grouped the maximum number of accessions (18), followed by cluster I (9) and cluster III (7). In the PCoA, the first three principal coordinates cumulatively explained 41.96% of the variability present in the accessions. The first and second principal coordinates explained 22.51% and 10.39% of the molecular variability, respectively (Table 7; Fig. 4). In general, both Nei's genetic distance-based cluster and PCoA showed wider variability within the groups. Based on the 17 SSR markers, the model based genetic structure analysis detected the maximal ΔK (80.87) at $K = 2$ (Supplementary Table 2), indicating that the entire germplasm collection could be divided

into two subgroups (Fig. 5). Out of 34 Job's tears accessions seven were admixtures. A total of 14 accessions showed more than 80% ancestry to the first sub-group (displayed as red) and 13 to the second sub-group (displayed as green).

Table 7
PCoA in Job's tear accessions

Percentage of variation explained by the first three coordinates			
Coordinate	1	2	3
%	22.51	10.38	9.06
Cum %	22.51	32.90	41.96

The two sub-groups generated from the structure analysis were analyzed for genetic variation among and within the sub-groups using AMOVA. From the analysis, 47.14% of the variation was observed among individuals, while 52.86% was observed within individuals (Table 8). The estimation of Wright's F statistic indicated that the F_{IS} for all 17 SSR loci was 0.47. The F_{ST} for the polymorphic loci across all accessions was 0.20, implying a high genetic variation (>0.15) (Table 8). The pairwise F_{ST} estimates among sub-groups indicated that the different groups formed based on the geographical locations of the accessions were significantly different from each other (Fig. 6). It was evident that the accessions of Mizoram and Assam were highly differentiated, followed by the accessions of Mizoram and Arunachal Pradesh. However, some extent of similarity was observed among the accessions of Nagaland and Meghalaya, Arunachal Pradesh and Meghalaya, and Nagaland and Arunachal Pradesh. The Mantel test showed no significant correlation between genetic and morphological distances ($r^2 = 0.010$) with a p-value of 0.41.

Table 8
AMOVA in Job's tear accessions

Source of variation	DF	Sum of squares	Variance	Percentage of variance
Among individuals	33.00	275.59	2.68	47.14
Within individuals	34.00	102.00	3.00	52.86
Total	67.00	377.59	5.68	
$F_{IS} = 0.47, F_{ST} = 0.20$				

Stability analysis for the yield and yield-related traits

The analysis of variance of additive main effects and multiplicative interactions (AMMI) (Table 9) showed that the mean sum of squares due to accessions, environments, and genotype-environment interactions were significant ($p < 0.01$), indicating the diverse nature of accessions (Vijayakumar et al. 2001). The significance of the environments indicated the distinctness of intrinsic factors in different environments (Table 9). Since the genotype-environment ($G \times E$) interactions were also significant, it can be inferred that the accessions exhibited differential performance in different environments. The sum of squares due to the $G \times E$ interaction was further partitioned into principal components and residual variation. For days to 50% flowering, PC1 and PC2 explained 58.10 and 41.90% of the variation, respectively. Likewise, the contribution of PC1 and PC2 (indicated in parenthesis) for days to 80% maturity (63.50, 36.50), plant height (71.90, 28.10), number of tillers per plant (58.20, 41.80), panicle length (62.50, 37.50), leaf length (67.90, 32.10), number of internodes per plant (67.20, 32.80), test weight (75.60, 24.40), and yield per plant (76.30, 23.70) towards $G \times E$ interactions were calculated, and it was found that the first two principal components could explain 100% of the $G \times E$ variation. The first principal component factor had a large contribution to the interaction sum of squares. This indicated that one fundamental factor affects $G \times E$ interaction; this could be either genotypic or environmental in nature.

Table 9
Additive main effect and multiplicative interaction analysis (AMMI) analysis of variance for yield attributing traits in Job's tear

Source	Df	DOF		DOM		PH		NT		PL	
		MSS	Proportion	MSS	Proportion	MSS	Proportion	MSS	Proportion	MSS	Propo
ENV	2	131.51**		228.73***		141830.29***		83.53***		20843.87***	
REP(ENV)	6	15.92***		4.21***		1089.05***		0.76		10.53	
GEN	33	42.85***		4.55***		2573.95***		5.03***		140.30***	
GEN:ENV	66	27.14***		4.45***		1240.63***		3.47**		114.57***	
PC1	34	30.59***	58.10	5.48***	63.50	1730.96***	71.90	3.93***	58.20	139.00***	62.50
PC2	32	23.48***	41.90	3.35***	36.50	719.65***	28.10	2.99	41.80	88.60***	37.50
Residuals 1	198	0.90		0.70		190.97		2.12		30.42	
Total	371	14.91		3.66		1554.47		3.28		182.01	
Source	Df	LL		Nis		TW		YPP			
		MSS	Proportion	MSS	Proportion	MSS	Proportion	MSS	Proportion		
ENV	2	9392.66***		12.72**		148.65***		163461.74***			
REP(ENV)	6	10.74		1.53*		0.33		295.81			
GEN	33	199.13***		3.90***		82.52***		2402.11***			
GEN:ENV	66	178.19***		2.94***		40.39***		1865.37***			
PC1	34	234.91***	67.90	3.84***	67.20	59.25***	75.60	2764.09***	76.30		
PC2	32	117.93***	32.10	1.99***	32.80	20.34***	24.40	910.49**	23.70		
Residuals 1	198	36.16		0.66		0.27		532.71			
Total	371	151.22		1.84		22.66		2047.64			

AMMI 1 and AMMI 2 biplot analysis

In the AMMI model I biplot, the PC1 scores of accessions and environments are plotted against mean values, which helps to visualize the average performance of the accessions and environments along with their interactions. Accessions or environments on the right side of the midpoint of the perpendicular line exhibited better performance than those on the left side. The accessions, namely, IC147053, IC419466, IC416897, and IC540279, had a higher test weight. In contrast, IC604098, IC540181, IC334314, and IC601106 had low test weights (Fig. 7a). Likewise, the accessions, namely, IC12703; IC334314; and 486143, had a larger panicle length (Fig. 8a). Regarding the number of tillers per plant, IC416831, IC89390, IC12703, and IC540222 exhibited better performance (Fig. 9a). For yield per plant, accessions, namely IC540181, IC89393, and IC89392, with large PC1 scores, were the best performing ones (Fig. 10a). Accessions with high and stable performance, as indicated by a PC1 score of zero or nearly zero, were IC600638, IC540181, IC540256, IC540279, and IC486143. These accessions exhibited a lower susceptibility to environmental influences. Thus, the AMMI biplot clearly indicated that the studied accessions differed from each other, not only for mean yield but also for their interaction effects.

The magnitude of interaction can be visualized for each accession and environment using the IPCA1 vs. IPCA2 biplot model (Yan and Hunt 1998). According to the AMMI II biplot, the accessions (and environments) that are located far away from the origin are more responsive to certain environments. Accordingly, IC417053, IC540279, and IC540181 for test weight (Fig. 7b), IC12703, IC540222, IC419466, and IC89394 for panicle length (Fig. 8b), IC416831, IC374506, IC604159, and IC416884 for the number of tillers per plant (Fig. 9b), were identified as high-performing accessions. Regarding yield per plant, IC416884, IC89393, and IC89387 for the year 2018, IC89393, IC89392, and IC540173 for the year 2019, and IC416897, IC417053, and IC540222 for the year 2022 were identified as promising, while IC540181, IC540256, IC600638, IC540181, and IC540392, were found promising in both the years 2019 and 2020 (Fig. 10b).

The AMMI stability value (ASV), the sum of absolute values of the IPC scores (SIPC), the average of the squared eigenvalue (EV), the absolute value of the relative contribution of IPCs to interactions (ZA), and the weighted average of absolute scores (WAAS) are used to evaluate the stability of a genotype. A lower value for these parameters indicates higher stability. In the present study, (1) ASV ranged from 0.285 to 4.130 for the number of tillers per plant, 0.239 to 7.660 for the test weight, and 0.305 to 14.700 for the yield per plant; (2) SIPC ranged from 0.273 to 4.000 for the number of tillers per plant, 0.208 to 4.010 for the test weight, and 0.100 to 6.810 for the yield per plant; and (3) EV ranged from 0.001 to 0.115 for the number of tillers per plant, 0.001–0.199 for the test weight, and 0.000–0.087 for the yield per plant; (4) ZA ranged from 0.026–0.336 for the number of tillers per plant, 0.019–0.461 for the test weight, and 0.006–0.313 for the yield per plant, and (5) the WAAS ranged from 0.151 to 2.030 for the number of tillers per plant, 0.082 to 2.220 for the test weight, and 0.073 to 3.980 for the yield per plant (Table 10).

Table 10
AMMI stability analyses for yield attributing traits in in Job's tear accessions

Accession	NT					TW					YPP				
	ASV	EV	SIPC	ZA	WAAS	ASV	EV	SIPC	ZA	WAAS	ASV	EV	SIPC	ZA	WAAS
IC22156	0.998	0.005	0.615	0.061	0.380	1.400	0.008	0.793	0.088	0.417	3.570	0.008	2.060	0.085	1.050
IC374506	2.820	0.065	2.970	0.246	1.470	1.990	0.012	0.967	0.115	0.561	13.400	0.049	4.520	0.247	3.250
IC416829	2.620	0.034	2.010	0.185	1.140	2.060	0.042	1.600	0.151	0.686	7.340	0.087	5.810	0.203	2.390
IC416831	1.830	0.018	1.540	0.138	0.843	0.497	0.001	0.242	0.029	0.140	13.600	0.055	5.190	0.265	3.450
IC416868	0.285	0.001	0.327	0.026	0.151	0.821	0.004	0.536	0.055	0.258	5.690	0.012	2.580	0.120	1.530
IC416884	1.950	0.057	2.220	0.162	0.929	7.660	0.199	4.010	0.461	2.220	6.630	0.084	5.540	0.188	2.190
IC416897	0.960	0.012	1.120	0.085	0.492	4.720	0.046	1.730	0.240	1.200	14.000	0.066	5.930	0.286	3.670
IC417053	1.190	0.007	0.910	0.084	0.517	5.250	0.098	2.800	0.319	1.530	10.600	0.044	4.910	0.225	2.860
IC419466	1.300	0.018	1.480	0.117	0.687	4.160	0.038	1.650	0.219	1.090	2.960	0.003	1.090	0.057	0.740
IC486143	2.580	0.033	1.990	0.183	1.130	2.780	0.016	0.985	0.139	0.699	2.730	0.002	1.050	0.053	0.692
IC521341	4.130	0.108	3.910	0.336	2.030	4.820	0.050	1.880	0.252	1.250	1.640	0.001	0.570	0.031	0.403
IC540173	2.260	0.060	2.610	0.203	1.190	0.598	0.012	0.618	0.041	0.163	9.310	0.027	3.750	0.186	2.400
IC540181	0.427	0.001	0.425	0.036	0.216	1.230	0.014	0.941	0.089	0.407	0.826	0.000	0.308	0.016	0.207
IC540222	1.860	0.043	2.170	0.167	0.974	0.783	0.012	0.750	0.062	0.270	12.800	0.051	5.070	0.253	3.280
IC540250	1.470	0.013	1.330	0.116	0.706	3.020	0.028	1.500	0.177	0.857	7.160	0.039	4.380	0.175	2.150
IC540256	0.427	0.002	0.495	0.038	0.224	0.634	0.001	0.208	0.031	0.156	1.870	0.009	1.700	0.055	0.625
IC540279	1.490	0.011	1.150	0.105	0.649	3.880	0.052	2.050	0.235	1.130	1.260	0.003	1.090	0.036	0.419
IC600638	0.440	0.001	0.273	0.027	0.168	1.720	0.078	1.770	0.136	0.571	1.530	0.012	1.530	0.037	0.364
IC600837	0.995	0.008	1.040	0.086	0.516	2.500	0.013	0.965	0.130	0.649	5.300	0.032	3.750	0.139	1.670
IC601106	0.841	0.007	0.953	0.076	0.446	1.120	0.028	1.110	0.089	0.381	8.490	0.020	2.690	0.152	2.020
IC604098	0.867	0.005	0.845	0.072	0.434	0.541	0.010	0.556	0.037	0.145	6.560	0.024	3.570	0.151	1.880
IC604098-1	3.460	0.054	2.220	0.215	1.350	0.239	0.001	0.220	0.019	0.082	4.220	0.006	1.860	0.088	1.120
IC604159	0.812	0.006	0.886	0.072	0.428	2.870	0.019	1.220	0.156	0.770	0.305	0.000	0.100	0.006	0.073
IC89383	0.812	0.006	0.886	0.072	0.428	3.480	0.032	1.610	0.197	0.963	14.700	0.084	6.810	0.313	3.980
IC89384	2.440	0.029	1.890	0.173	1.070	1.600	0.015	1.030	0.107	0.501	1.080	0.005	1.130	0.031	0.333
IC89387	3.910	0.080	3.240	0.291	1.780	1.530	0.007	0.772	0.090	0.437	4.850	0.035	3.740	0.133	1.570
IC89390	0.895	0.013	0.992	0.071	0.402	0.695	0.002	0.361	0.042	0.201	10.600	0.032	3.840	0.201	2.630
IC89391	0.799	0.003	0.547	0.052	0.324	1.290	0.007	0.759	0.082	0.390	7.290	0.042	4.540	0.180	2.200
IC89392	1.660	0.028	1.860	0.149	0.880	1.130	0.009	0.770	0.078	0.362	10.300	0.054	5.390	0.233	2.910
IC89393	0.554	0.002	0.524	0.045	0.273	0.530	0.001	0.318	0.034	0.161	5.500	0.081	5.090	0.162	1.840
IC89394	3.920	0.115	4.000	0.335	2.010	2.000	0.065	1.810	0.156	0.689	9.560	0.027	3.650	0.186	2.420
IC12703 ©	3.370	0.112	3.770	0.302	1.790	4.670	0.065	2.290	0.272	1.320	1.120	0.002	0.854	0.031	0.362
IC334314 ©	1.780	0.016	1.430	0.130	0.797	1.520	0.013	0.979	0.102	0.476	0.575	0.001	0.453	0.016	0.187
IC521338 ©	2.340	0.026	1.710	0.160	0.989	0.317	0.003	0.332	0.023	0.092	0.883	0.004	0.903	0.022	0.226
Min	0.29	0.00	0.27	0.03	0.15	0.24	0.00	0.21	0.02	0.08	0.31	0.00	0.10	0.01	0.07
Max	4.13	0.12	4.00	0.34	2.03	7.66	0.20	4.01	0.46	2.22	14.70	0.09	6.81	0.31	3.98

Factors linked to correlated traits, selection differential, response to selection and indicators

The traits, namely, days to 50% flowering and days to 80% maturity, were negative for selection differential and thus response to selection. The factor analysis revealed that traits like plant height, panicle length, leaf length, number of internodes per plant, and test weight are responsive to selection under the conditions prevalent in Meghalaya. Also, higher uniqueness values for days to 50% flowering, days to 80% maturity, panicle length, and test weight showed that these

traits are affected by unique factors that are not related to common factors (Table 11). Moreover, the traits, such as the number of tillers per plant, leaf length, number of internodes per plant, and test weight exhibited higher communality and lower uniqueness, indicating that a common factor explains the variance for these traits and is highly effective in accounting for total variations compared to other traits.

Table 11
Factors linked to correlated traits, selection differential, response to selection and indicators for traits in job's tear

Variables	FA1	FA2	Communality	Uniqueness	Xo	Xs	SD	SDperc	Sense	Goal
DOF	-0.58	-0.21	0.37	0.63	84.5	83.4	-1.08	-1.27	increase	0
DOM	0.73	0.01	0.53	0.47	158	157	-0.878	-0.556	increase	0
PH	-0.2	0.68	0.5	0.5	206	211	4.64	2.25	increase	100
NT	-0.75	-0.3	0.65	0.35	4.39	5.2	0.812	18.5	increase	100
PL	0.69	-0.32	0.57	0.43	45	46	0.997	2.21	increase	100
LL	0.67	-0.46	0.67	0.33	49.5	54.2	4.69	9.48	increase	100
Nls	0.76	-0.23	0.63	0.37	6.92	6.96	0.0345	0.499	increase	100
TW	0.69	0.76	0.59	0.41	12	15.4	3.44	28.8	increase	100
YPP	0.25	-0.8	0.7	0.3	58.6	77.8	19.2	32.7	increase	100

Association among stability parameters and multi-trait stability index (MTSI)

All the stability measures showed a significant positive correlation with each other (> 83%). We conducted a multi-trait stability index (MTSI) analysis to identify the accessions that exhibited stability for all the yield-attributing traits within each environment. Based on MTSI, out of 34 accessions, five, namely, IC600638, IC89392, IC540181, IC604098, and IC540256, were found suitable for selection at 10% selection intensity for yield-related traits. These accessions crossed the cut-off point (red circle), as presented in Fig. 11. These accessions were compared with the mean performance based on all the traits and were found to be promising and stable in nature.

Discussion

The northeastern region of India is known to harbour a rich diversity of Job's tears germplasm. However, only minimal efforts have been made to assess the diversity which is crucial to ensure that the most diverse genotypes are selected to widen the genetic base of the germplasm and to integrate favourable alleles for successful crop improvement (Sharma et al. 2021). In the present study, we aimed to analyse the genetic diversity in 34 diverse Job's tears accessions, collected across the Northeastern Himalayan states of India, at the morphological and molecular level *vis-à-vis* identification of promising Job's tears accessions with high and stable performance in Northeast India.

Yield is the most crucial and complex trait in crops. It reflects an interplay of environmental factors with growth and development processes throughout the crop's life cycle (Quarrie et al., 2006). In this sense, splitting the variations in traits into genotypic and environmental components is crucial (Zaid et al. 2022). The study observed a higher PCV compared to the GCV for all nine yield and yield-related traits considered for the study. This suggests a substantial influence of environmental factors on trait variations in Job's tears. The heritability of a plant trait is crucial for assessing its transmissibility to future generations (Surek and Beser, 2003). Traits with high genetic advance and high heritability offer optimal conditions for selection in breeding programs (Larik and Rajput, 2000). Notably, test weight and leaf length demonstrated high heritability along with a high genetic advance over the mean, indicating that these traits are governed by additive gene action, making their selection advantageous. Conversely, traits like days to 80% maturity, panicle length, and number of tillers per plant exhibited low heritability and low genetic advance over the mean, suggesting a significant influence of the environment on these traits and rendering selection less effective. Yield per plant and days to 50% flowering showed low heritability but high genetic advance over the mean, indicating potential effectiveness in selection, with low heritability likely attributed to environmental influences on trait expression.

The PCA serves to simplify the analysis of multiple indices by transforming original and correlated indices into new ones. Previous research has highlighted the effectiveness of PCA in comprehensive crop evaluation (Wang et al., 2013). In the present study, the PCA method was employed to comprehend the information from nine yield and yield-related traits of 34 Job's tears accessions into three main components, simplifying the data and unveiling relationships between variables. Regarding the nature of trait variations, the degree of trait contributions in the first three principal components was crucial (Clifford and Stephenson 1976). These components can serve as a valuable scientific foundation for parent selection in breeding programs (Li et al., 2015). Breeders can capitalize on these key factors and diversify the traits of breeding materials to expedite breeding programs for the development of new varieties.

A thorough understanding of genetic diversity and the association of yield-contributing traits with yield per plant is crucial to a breeding programme aimed at enhancing yield (Katema and Geleta 2022). Path analysis is an appropriate statistical technique for examining and testing relationships among observed variables. Furthermore, these relationships or paths may represent direct or indirect associations. In the present study, the path coefficient analysis revealed a residual effect (0.32). This implies that the nine quantitative traits analyzed in the study accounted for 99.68% of the total variation in Job's tears germplasm accessions. Test weight and number of internodes per plant exerted a very high positive direct effect on yield per plant, indicating that selecting these traits would directly help improve yield in Job's tears.

Genetic diversity in crop germplasm provides an opportunity for plant breeders to analyse and establish genetic relationships among the germplasm accessions and monitor and identify diverse parental combinations to create segregating progenies (Ramadan et al. 2015). Plant breeders can assess the

genetic diversity by using various multivariate statistical methods. It helps understand the extent of genetic diversity by unveiling the complex interactions between genotypic and phenotypic traits and factors affecting crop diversity. Cluster analysis and PCA are the most commonly used multivariate statistical diversity assessing methods in agriculture. Cluster analysis can be done with morphological and molecular data alone or in combination, with the latter being more reliable (David and John 2000). If done individually, the molecular approach has been found to be more robust and effective, as morphological traits are easily affected by environmental conditions and cultural practices. In the present study, clustering based on nine yield and yield-related traits grouped 34 Job's tears accessions into four major clusters. Cluster I grouped all the short-height accessions, while cluster II grouped the accessions with higher test weights. Cluster III consisted of accessions with high yields, while cluster IV grouped the accessions of early flowering types. The trait-based clustering observed in the study highlights the efficacy of the clustering approach in resolving the germplasm accessions in Job's tears. Based on the PCA analysis, the present study observed that traits like days to 50% flowering, plant height, leaf length, number of internodes per plant, test weight, and yield per plant are crucial for distinguishing Job's tears accessions. The molecular-based approach, being more effective, has been followed by several workers for estimating the genetic diversity in Job's tears germplasm (Li et al. 2001; Ma et al. 2010; Wang et al. 2015; Fu et al. 2019). In the present study, using 17 SSR markers, 34 Job's tears accessions could be resolved into three major clusters based on Nei's genetic distance. The PCoA based on SSRs substantiated the SSR-based clustering of Job's tears accessions.

Multiple evolutionary factors, including the breeding system, gene flow (pollen and seed dispersal), life form, and geographic range (Xi et al. 2016), affect the genetic structure of a species. Model-based population structure in line with clustering based on genetic distance indicated that the 34 Job's tears germplasm accessions could be divided into two subgroups based on the maximal ΔK value. Similar model-based genetic clustering of Job's tears accessions has also been reported earlier (Li et al. 2001; Ma et al. 2010; Wang et al. 2015; Fu et al. 2019). Among the 17 SSR markers employed for diversity analysis, the present study found GBssrJT149 to be the most informative marker. It can be helpful in identifying distant Job's tears parental lines for various crop improvement programmes. The analysis of the genetic parameters using 17 SSR markers indicated that the mean value (0.28) of observed heterozygosity (H_o) was less than the corresponding mean value (0.44) of expected heterozygosity (H_e). This implies that the population under study is inclined more towards inbreeding than random mating or outcrossing. This could be due to the sample sources, population size of the collection, and human activities of long-term domestication and selection over the years. The present study observed that the Shannon's Diversity Index (I) value of 0.06 to 1.04 obtained in the present study was comparatively higher than the earlier report of 0.28 to 0.39 from the study of 139 Job's tears genotypes from Southwest China by Fu et al. (2019). A significant differentiation among accessions of Mizoram and Assam, followed by accessions of Mizoram and Arunachal Pradesh, could be due to geographical isolation. There has not been gene flow from Mizoram to the other states due to geographical barriers. Similarity among the accessions of Nagaland and Arunachal Pradesh with Meghalaya has been observed, stating that the genotypes from these regions have been mixed at a certain point in time. The non-congruence of clustering patterns against their geographical place of collection with low genetic variations among the Job's tears genotypes has been reported earlier by several workers (Li et al. 2001; Wang et al. 2015; Xi et al. 2016; Fu et al. 2019).

The present study revealed that clustering based on morphology and SSRs was different. Earlier studies in various other crops have also observed a low correlation between morphology and SSR-based clustering, consistent with our results (Wang et al. 2023). According to Li et al. (2018), both innate and environmental factors affect morphological traits, contributing to the complexity of this phenomenon. In contrast, DNA markers indicate differences in DNA sequence and are mostly present in noncoding regions.

The stability of Job's tears germplasm accessions was assessed using the Additive Main Effects and Multiplicative Interaction (AMMI) model. The AMMI model is advantageous as it provides overall fitting, imposes no constraints on multiplicative terms, and yields the least-square fit (Freeman 1990). The considerable sum of squares for accessions in this study indicates the diversity among accessions, with significant differences among genotypic means being the primary cause of variation in grain yield. This aligns with previous research findings (Misra et al. 2009).

The significance of the environments indicated the distinctness of intrinsic factors in different environments. As the $G \times E$ interactions were also significant, it can be stated that the accessions performed differentially in various environments. The sum of squares due to the $G \times E$ interaction was further partitioned into principal components and residual variation. For yield per plant, PC1 and PC2 explained 76.30% and 23.70% of total $G \times E$ interactions, thus explaining 100% of the $G \times E$ variation. Among the 34 Job's tears, IC600638, IC89392, IC540181, and IC540256 were identified as promising and stable and could be effectively used in the breeding programmes. Several parameters of AMMI were analyzed, and the superiority of accessions, as depicted in the AMMI biplot, was re-confirmed. Based on MTSI, five accessions, namely, IC600638, IC89392, IC540181, IC604098, and IC540256, were identified at 10% selection intensity for yield and its component traits.

Conclusion

This study characterized 34 Job's tears accessions from the Northeastern Himalayan region using yield-related traits and SSR markers. Genotyping with 17 SSR markers revealed an average of 3.18 alleles per locus. Molecular analyses, including clustering and PCoA, identified three major clusters and two subgroups, emphasizing underlying genetic variations. AMOVA highlighted substantial genetic diversity within individuals, with notable population differentiation, particularly in Mizoram and Assam. Morphological trait analysis categorized accessions into four, each with a dominating morphological trait. Positive correlations, especially with test weight, were observed, and path coefficient analysis indicated the direct positive effects of test weight and the number of internodes per plant on yield. While consistent patterns were observed in cluster analysis, the Mantel test indicated no significant genetic-morphological correlation. AMMI analysis and MTSI identified three promising accessions, IC600638, IC540181, and IC540256, holding potential for future breeding programs at a 10% selection intensity.

Declarations

Authors' contributions

AK conceived the project and wrote the manuscript. AK, SPD, AP designed the experiments. AK, SPS, PWS, LT performed the experiments. SK, SJ, VKV provided valuable inputs in the research work. AK, AP, BKS analyzed the data. BKS, VKM coordinated the project and edited the manuscript. All the authors read and approved the manuscript.

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Competing Interest

The authors of this manuscript have no competing interests.

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Figures

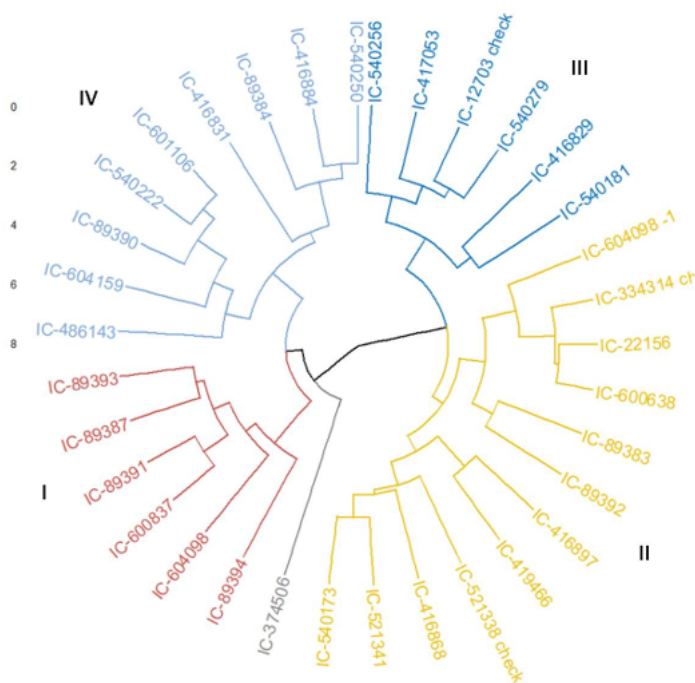


Figure 1

Clustering of Job's tear accessions based on yield and yield-related traits

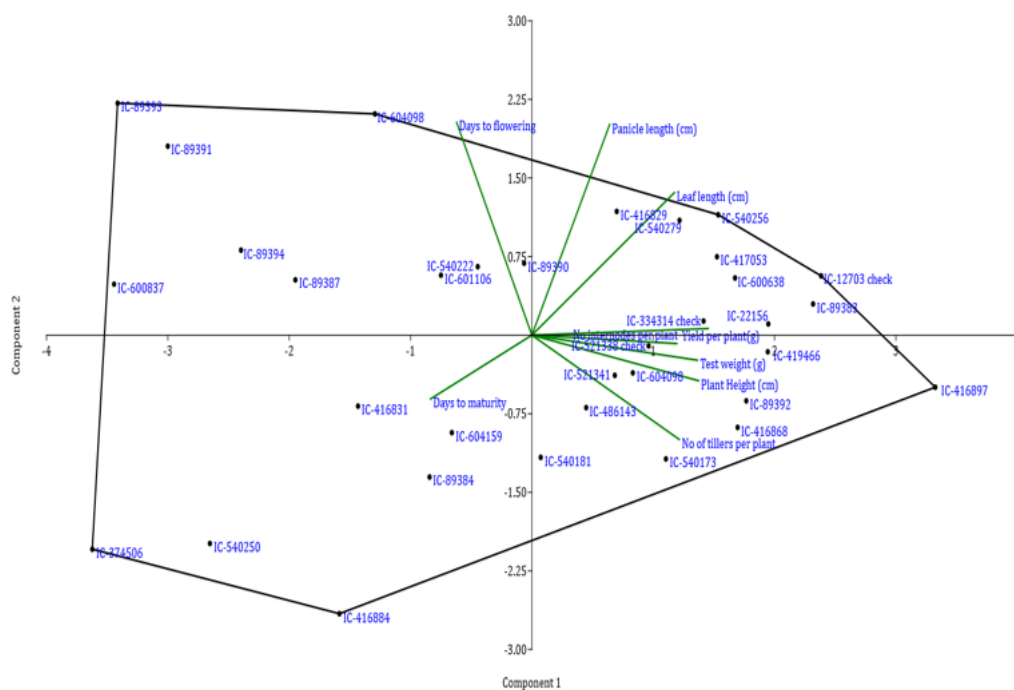


Figure 2
Genotype-by-trait (GT) biplot of Job's tear accessions based on yield and yield-related traits

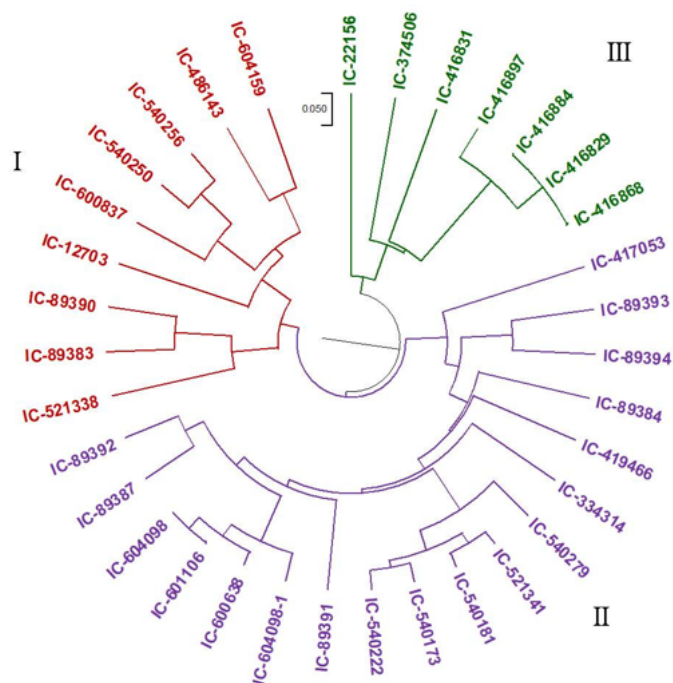


Figure 3
Clustering of Job's tear accessions following UPGMA cluster analysis according to Nei's genetic distance

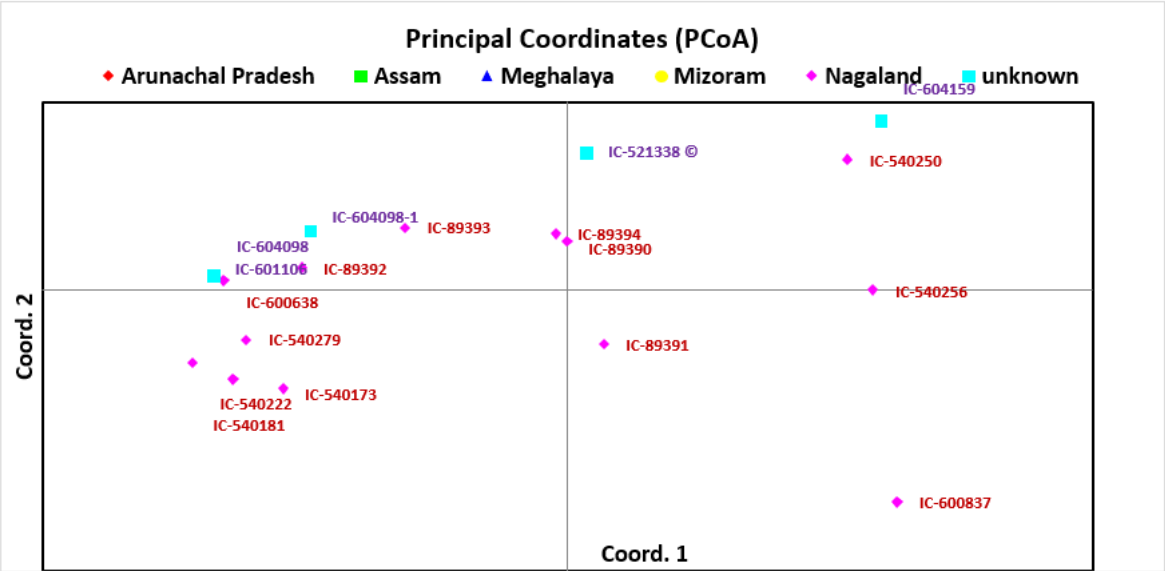


Figure 4

PCoA in Job's tear accessions

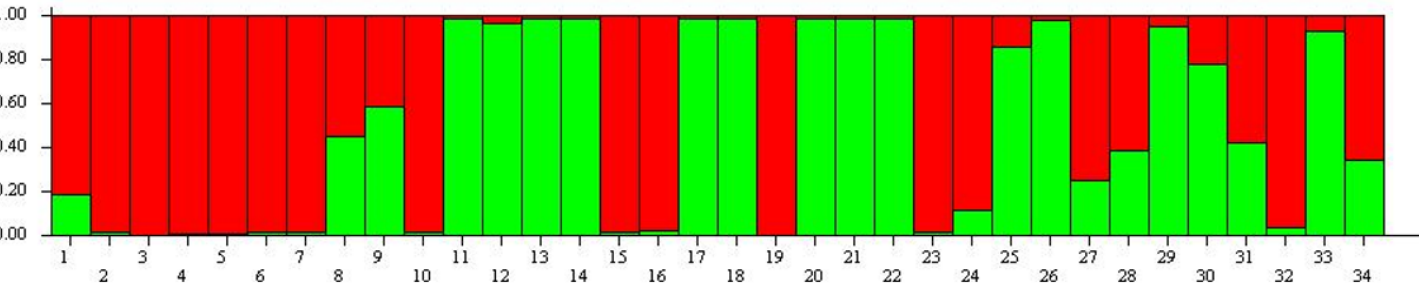


Figure 5

Model-based clustering of Job's tear accessions

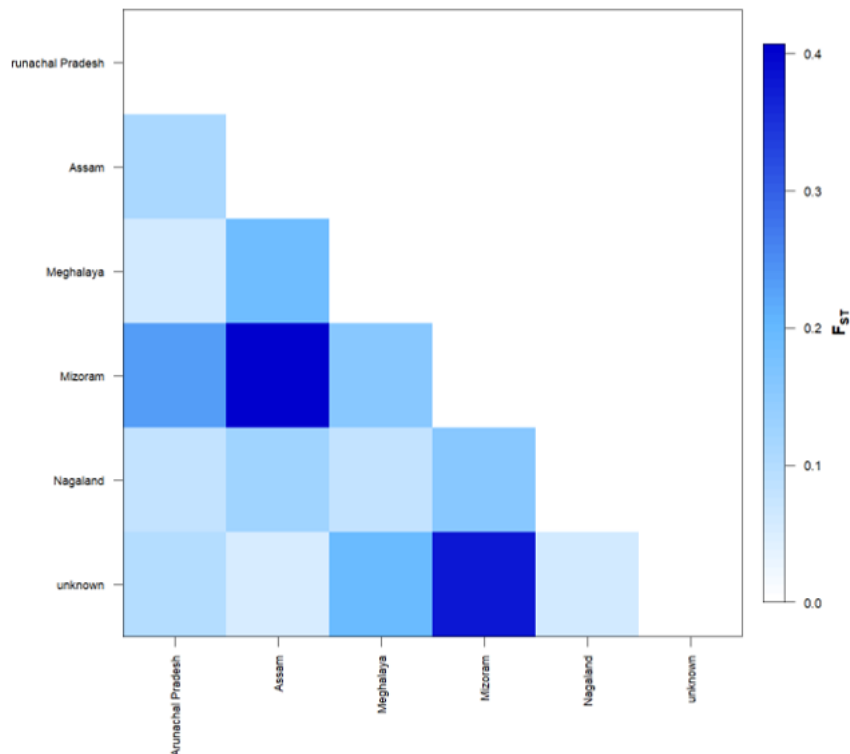


Figure 6

Pairwise population differentiation according to groups of populations in Job's tear accessions

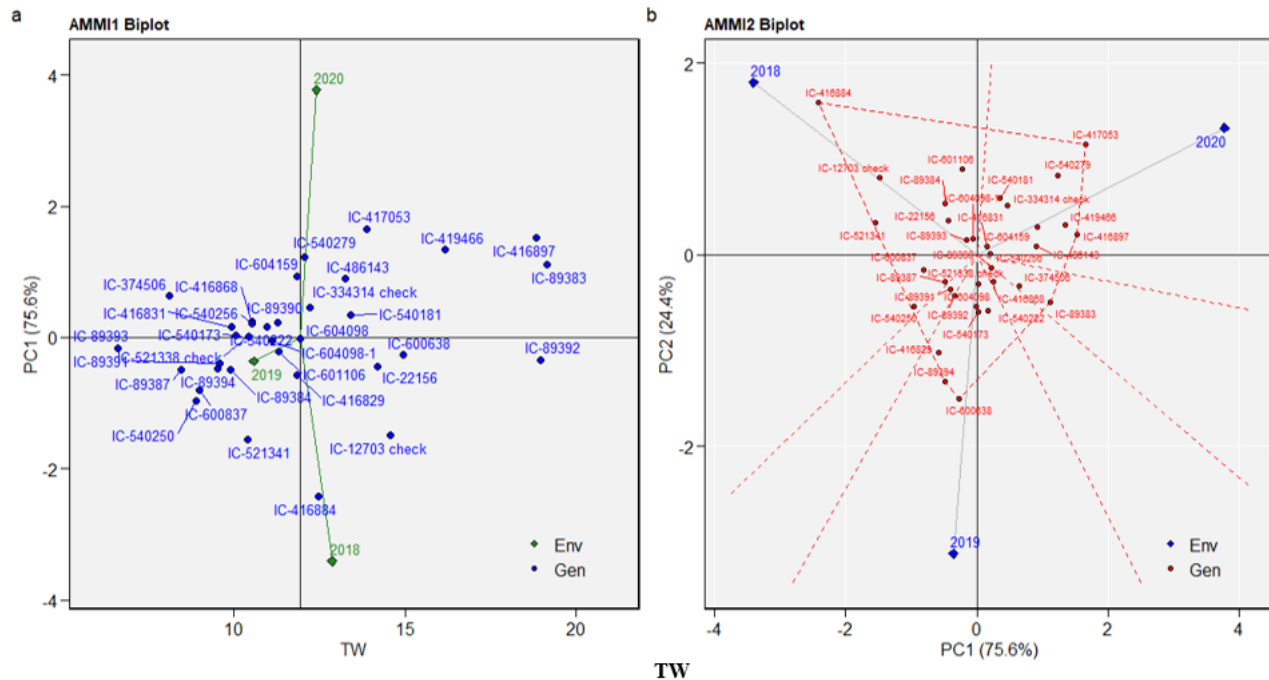


Figure 7

a AMMI 1 Biplot for test weight (TW) of 34 Job's tear accessions (G) and three years (E) using genotypic and environmental scores

b AMMI 2 Biplot for test weight (TW) of 34 Job's tear accessions (G) and three years (E) showing the interaction of IPCA2 against IPCA1 scores

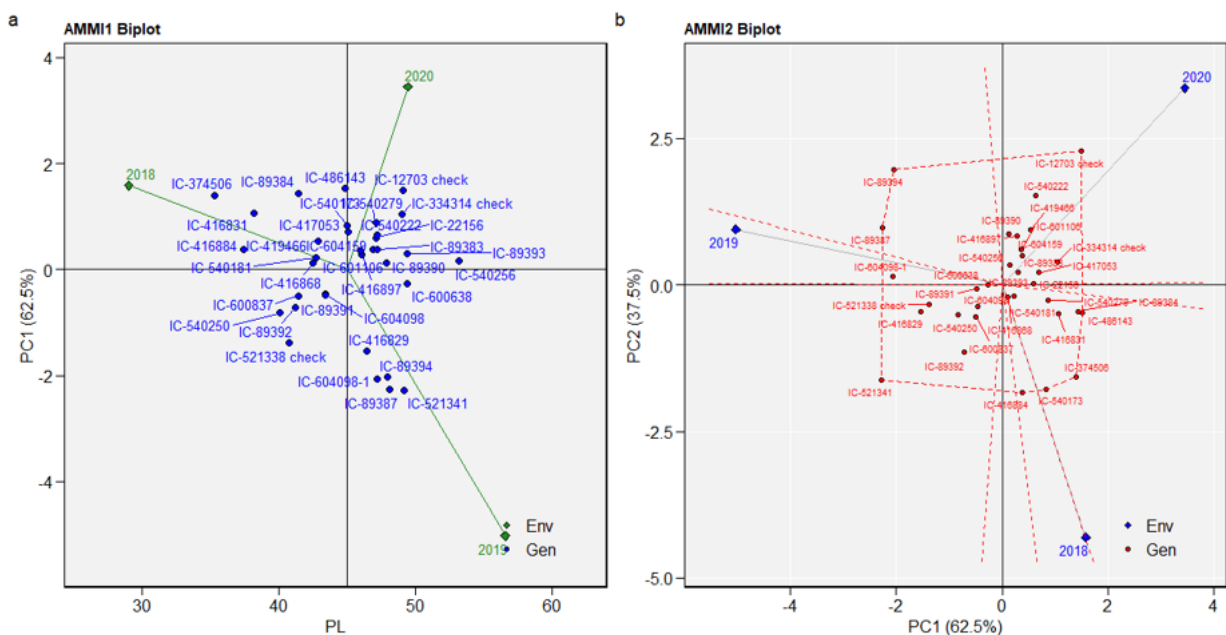


Figure 8

a AMMI 1 Biplot for panicle length (PL) of 34 Job's tear accessions (G) and three years (E) using genotypic and environmental scores

8b AMMI 2 Biplot for panicle length (PL) of 34 Job's tear accessions (G) and three years (E) showing the interaction of IPCA2 against IPCA1 scores

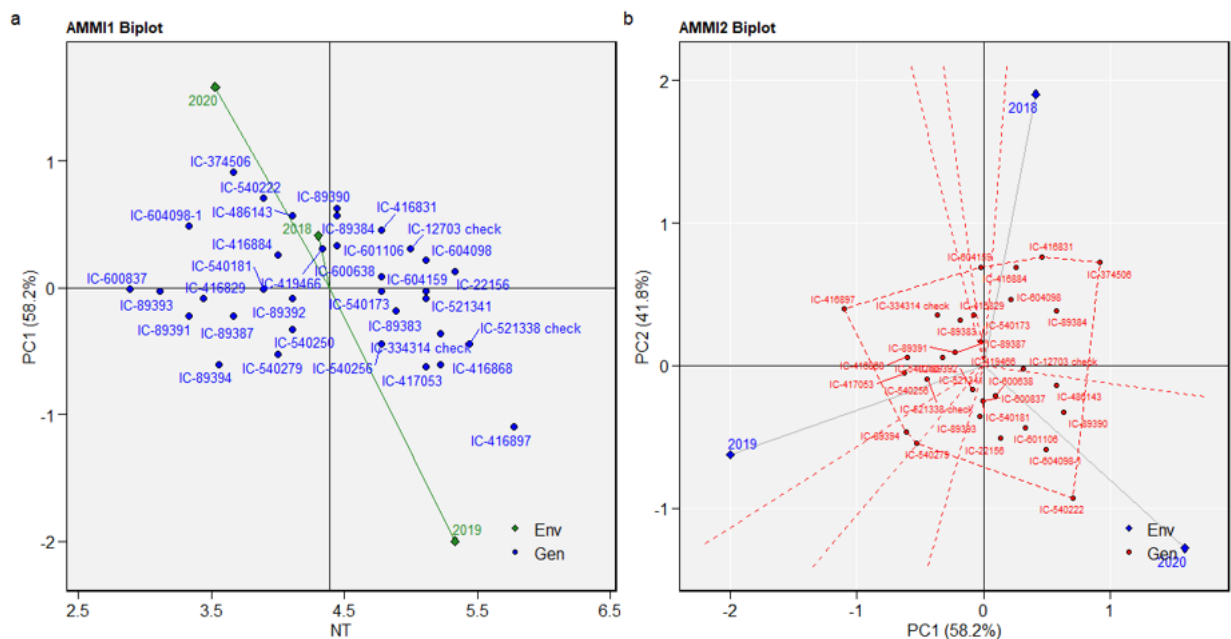


Figure 9

a AMMI 1 Biplot for number of tillers per plant (NT) of 34 Job's tear accessions (G) and three years (E) using genotypic and environmental scores

9b AMMI 2 Biplot for number of tillers per plant (NT) of 34 Job's tear accessions (G) and three years (E) showing the interaction of IPCA2 against IPCA1 scores

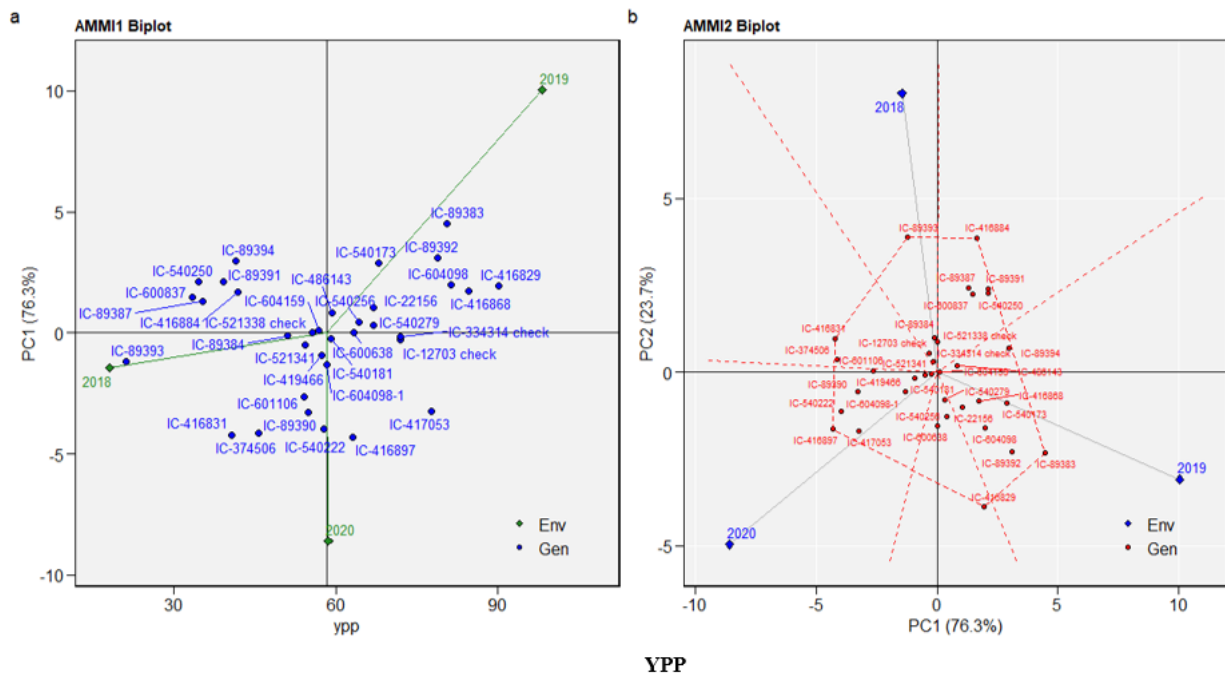


Figure 10

a AMMI 1 Biplot for yield per plant (YPP) of 34 Job's tear accessions (G) and three years (E) using genotypic and environmental scores

10b AMMI 2 Biplot for yield per plant (YPP) of 34 Job's tear accessions (G) and three years (E) showing the interaction of IPCA2 against IPCA1 scores

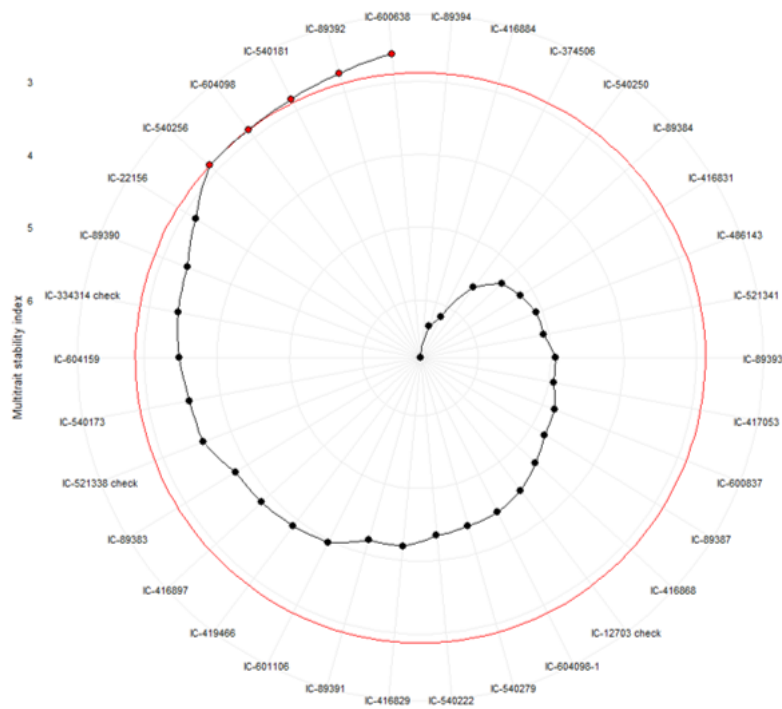


Figure 11

Genotype ranking in ascending order for the multi-trait stability index (MTSI)

Supplementary Files

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