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Genetic diversity in male and female landraces of teasel gourd in north-eastern India and strategies for crop improvement through induced monocliny

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Abstract

Background Teasel gourd is an important, indigenous, vegetatively propagated, high-value, underutilized cucurbit vegetable crop grown in South and Southeast Asia. Due to its wider adaptability, it is grown from plains to mid-hills. The crop is lacking in research, primarily related to the extent of genetic diversity in the region and crop improvement, which is further constrained by dioecism. To assess the genetic diversity in male and female populations of teasel gourd based on morphological traits and microsatellite makers and the response of $AgNO_3$ to induce monocliny, seventy genotypes, including eight males, were collected from different regions of the Northeastern states of India.

Results Under evaluation trials, wider variability was observed for leaf, flower, and fruit characteristics. Traits: ovary length ranged from 0.58 to 1.23 cm, fruit length 4.76 to 11.23 cm, fruit diameter 3.0 to 3.13 cm, fruit weight 22.8 to 129.3 g, and 100 seed weights 12.60 to 36.3 g, reducing sugar 2.99 to 7.39%, and vitamin-C content from 44.80 to 79.68 mg/100 g. The fruits and quality attributes have also shown high heritability (> 60%) and genetic advance (> 20%). Under molecular analysis, out of 43 microsatellite markers, 40 were polymorphic, and the polymorphism information content (PIC) ranged from 0.08 (Sed-09) to 0.68 (McSSR-5). A moderate genetic diversity was observed in the male and female genotypes based on gene diversity, PIC, and Nei's genetic distance. The additive main effects and multiplicative interaction (AMMI) analysis of variance for fruit traits has shown a significantly higher contribution of the genotype, followed by the genotype × environment interaction. Based on multi-trait stability index analysis for fruit traits, genotypes MNTGC-2, MNTGC-1, MNTGC-4, MZTGC-1, and ASTGC-3 were the most stable. Foliar application of AgNO3 at 500 mg l^{-1} was best for inducing hermaphroditism in female genotypes. The pollen germination can be enhanced to 82.3% over the control (23.1%) by the application of nutrient media comprised of sucrose (15%), boric acid (25 mg l^{-1}), and calcium nitrate (25 mg l^{-1}).

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Conclusions The available diverse genetic resources in teasel gourd can be effectively utilized by selecting stable superior genotypes, crop multiplication by selfing in superior induced hermaphrodite genotypes and exploiting heterosis by mating among diverse genotypes using silver nitrate.

Keywords Teasel gourd, *Momordica* spp., Genetic diversity, Hermaphroditism, Genetic stability

Background

Teasel gourd (*Momordica subangulata* subsp. *renigera*) is an essential Indigenous, perennial and dioecious cucurbit vegetable crop of *Momordica*. Among the *Momordica* species, the teasel gourd is allotetraploid (2n = 4x = 56) and originated as a segmental allopolyploid [1]. It is known by different names in local dialects, such as *Kakrol* (Hindi), *Bhat Karela* (Assamese), *Karkal* (Bengali), *Kokoda* (Marathi), kartoli, etc. The tender fruits are used for cooking vegetables and stuffing with spices. The crop is also a rich source of several nutrients, such as protein, β-carotene, vitamin C, and mineral iron [2]. The leaves, fruits, and seeds of *Momordica* spp. are also known for medicinal properties like anti-diabetic, anti-cancerous, anti-bacterial, and antiviral activities [3–5]. Due to widespread adaptability, it is grown in tropical and subtropical parts of India and other South and South East Asian countries like Myanmar, Bangladesh, etc. Although teasel gourd is considered an underutilised crop at the country level, it is grown in the entire Northeastern region of India in backyards and extensively for the markets in West Bengal, Orissa, and the Brahmaputra and Barak valley of Assam, and Tripura under cucurbits (teasel gourd, bitter gourd, cucumber, bottle gourd, etc.) followed by legume (Dolichos bean) cropping system during February– March to July– August and August– September to January– February, respectively [5].

Although there are wide variations in plant leaf, flower, seed, and fruit characteristics, there is no information on the extent of genetic diversity of teasel gourds available in the region. Some researchers have studied diversity in other parts of the country [6].This species, which had been grown in the region, including Bangladesh, was previously considered *Momordica dioica* [7–10] and *Momordica cochinchinensis* Spreng. (Sweet gourd) by Sanwal et al. [11] is now ascribed as *Momordica subangulata* subsp. *renigera* [1]. Molecular markers, along with quantitative traits, are the most effective tools for the analysis of genetic diversity and have been utilised in many underutilised cucurbits from the region, such as *Momordica* spp., *Benincasa hispida*, and Sechium *edule* [12–14] using RAPD and ISSR markers. Simple sequence repeat (SSR) is a codominant marker, most reliable and effective for the genetic characterisation of germplasm, mapping of genes linked to desirable traits, and marker-assisted breeding. The microsatellite markers have been developed and successfully used in different *Momordica* spp [15]. Although wide variability has been observed in teasel gourd for both male and female types and found in the wild as well as cultivated in the region, there is also a lack of research work on genetic diversity specific to economically important native landraces of teasel gourd (*Momordica subangulata* subsp. *renigera*) in the region. Moreover, there is no research work on the genetic diversity among the native male genotypes, which is very important for the creation of variability in the population.

Quantitative traits like fruit length and weight are polygenic and highly influenced by the genotype (G), environment (E), and their dynamic interactions. Therefore, knowledge of G × E interactions is critical for identifying the stable genotypes suitable for the mega-environment or diverse environmental conditions. The Additive Main Effects and Multiplicative Interaction (AMMI) method is a popular statistical approach for genetically stability analysis, particularly well-suited for datasets with numerous ecological influences. This method provides a nuanced understanding of the genotype-by-environment interaction, elucidating the motif of relationships between genotypes and environments through precise trait estimates [16–18].The use of AMMI alongside multi-trait stability index (MTSI) analysis for key fruit characteristics in teasel gourd will aid in understanding the influences of G, E, and G × E interactions, as well as in identifying stable genotypes for various fruit traits simultaneously.

Due to the dioecious and perennial nature of the crop, it is commercially propagated through sprouted roots. The dioecism not only limits crop production by maintaining the unproductive male genotypes (10–15%) but also leads to crop improvement as it is governed by a single recessive gene [female, homozygous recessive (xx) and the male heterozygous (xy)], and segregates in a 1:1 ratio if grown from the seeds [11], making it hard to identify the female at an early stage. The sex expression in cucurbits is also highly influenced by plant growth regulators; exogenous application of $AgNO_3$ has been found effective for the induction of hermaphroditism in female genotypes of Kakrol (*Momordica dioca*) [19–21] and Sweet gourd (*Momordica cochinchinensis* Spreng.) for hybridisation between two female genotypes (homosexual) for crop improvement [11]. Moreover, no study has been conducted on crop relatives to *Momordica subangulata* subsp. *renigera*.

Assuring the successful genetic exchange, establishment, and survival of the species, pollen plays a crucial role in the evolutionary development of higher plants [22,

23]. In order to comprehend the physiological and biochemical mechanisms underlying these processes [24–26], as well as to screen for genotypes that are resistant to abiotic stresses, pollen viability and germination have been studied in several cucurbits [27, 28] and many other crop species [29, 30]. Besides, pollen is also an excellent model system for studying several essential developments in cell biology [31, 32]. Because of this, pollen studies are now among the most intriguing research topics in plant science. The studies on pollen are carried out directly, either under in vivo or in vitro conditions. Under in vivo conditions, pollen viability and germination depend on the species and environmental conditions. However, under in vitro conditions, it depends on the nutrients of the testing media. Identifying the suitable growing medium is essential to assess the extent of pollen germination under in vitro conditions, especially in chemically induced monoclinous flowers.

In the recent past, the area under teasel gourd has increased due to the higher market price, which ranges from ₹ 60–80 kg^{-1} in retail markets, and the demand for the crop has also gone up due to an increase in awareness among consumers for its nutraceutical properties. To conserve the genetic resources and identify elite lines, the present research work has been initiated with the following objectives: to study the genetic diversity and phylogenetic relationship among male and female accessions of teasel gourd based on agro-morphological traits and molecular markers; the effect of $AgNO_3$ on induction of monocliny in female genotypes; the influence of growing nutrient media on pollen germination; and the identification of the genetic stable teasel gourd genotypes for the fruit characteristics.

Materials and methods

Plant materials

Seventy diverse genotypes of teasel gourd comprising sixty-two females and eight males were collected from forty-eight locations in Northeastern states of India (Supplementary Table 1). Out of 70 genotypes, fifty-two females and seven males were collected from vegetative parts i.e., sprouted roots, while eleven genotypes including one male were of sexual origin from the seedling selection. The maximum number of collections (25) were from the Tripura (22 females and 3 males), followed by Assam (11 females and 2 males), Meghalaya (10 females and 2 males), Mizoram (9 females), Manipur (7 females and 1 male), Arunachal Pradesh (2 females) and Nagaland (one female). The sites of the collections were mapped (Fig. 1) using online software (https://qgis.org/en/site/).

Evaluation of yield and quality traits

To assess the genetic diversity in teasel gourd accessions of north-eastern India, a set of 62 female genotypes were evaluated for different quantitative traits from March to October (2021–2022) for two consecutive years. The experimental field was Horticulture Farm (latitude 25.41 N and 92.55E longitude, elevation 960 m above mean sea level) of the Division of System Research & Engineering, ICAR Research Complex for NEH Region, Umiam, Meghalaya. Yearly, rainfall at the site ranged from 2,200 to 2,551 mm, and the average maximum and minimum temperatures during the crop period were 28.6 °C (August) and 14.3 °C (April), respectively. The soil is sandy in texture (inceptisol) and acidic in reaction (5.4 pH). The sprouted root tubers were planted in the pits $60 \times 60 \times 60$ cm in size and 1.5×2.0 m spacing between plants and rows, respectively, in a randomised complete block design (RCBD) with three replications. The pits were filled with FYM (10 t ha^{-1}) and chemical fertiliser NPK applied at 80:60:60 kg ha^{-1} from urea, single superphosphate (SSP), and muriate of potash (MOP), respectively. The complete dose of P and K with half of N was applied in the pits during land preparation. The remaining N dose was applied equally in the ring basin 30 and 60 days after planting (DAP), followed by hoeing. The plants were trained on a trellis. The preventive measure to control pests, the systemic insecticide Rogor (30% EC) (1.0 ml l^{-1} water), was sprayed at 30 and 60 DAP. The observations for quantitative traits FFN: first flowering node, OL: ovary length (cm), SL: sepal length (cm), SW: sepal width (cm), PL: petal length (cm), PW: petal width (cm), FW: fruit weight (cm), FL: fruit length (cm), FD: fruit diameter (cm), STL: style length (cm), STD: style diameter (cm), PDL: pedicel length (cm), PDD: pedicel diameter (cm), NFPP: number of fruits/plant, YPP: yield per plant (kg), HSW: 100 seed weight (g), SDL: seed length (cm), SDW: seed width (cm), SDT: seed thickness (cm), LFL: leaf length (cm), and LFW: leaf width (cm) from six plants, flowers, fruits, and leaves was taken. To investigate the stability of fruit attributes and based on the availability of planting materials (sprouted roots), a set of 36 genotypes was chosen and assessed from March to October for the four consecutive years (2019–2022). Observations for the fruit traits, viz., FL: fruit length (cm), FD: Fruit diameter (cm) and FW: fruit weight (g) was recorded for the further analysis.

Quality analysis

The quality parameters of tender fruits, viz., total soluble solids (TSS), reducing sugar (RS), and vitamin C (VC) content were taken. TSS content was estimated using a digital refractometer (HI96801, Hanna Instruments, Romania). Reducing sugar content was estimated by following the Phenol-Sulfuric method described by Dubois

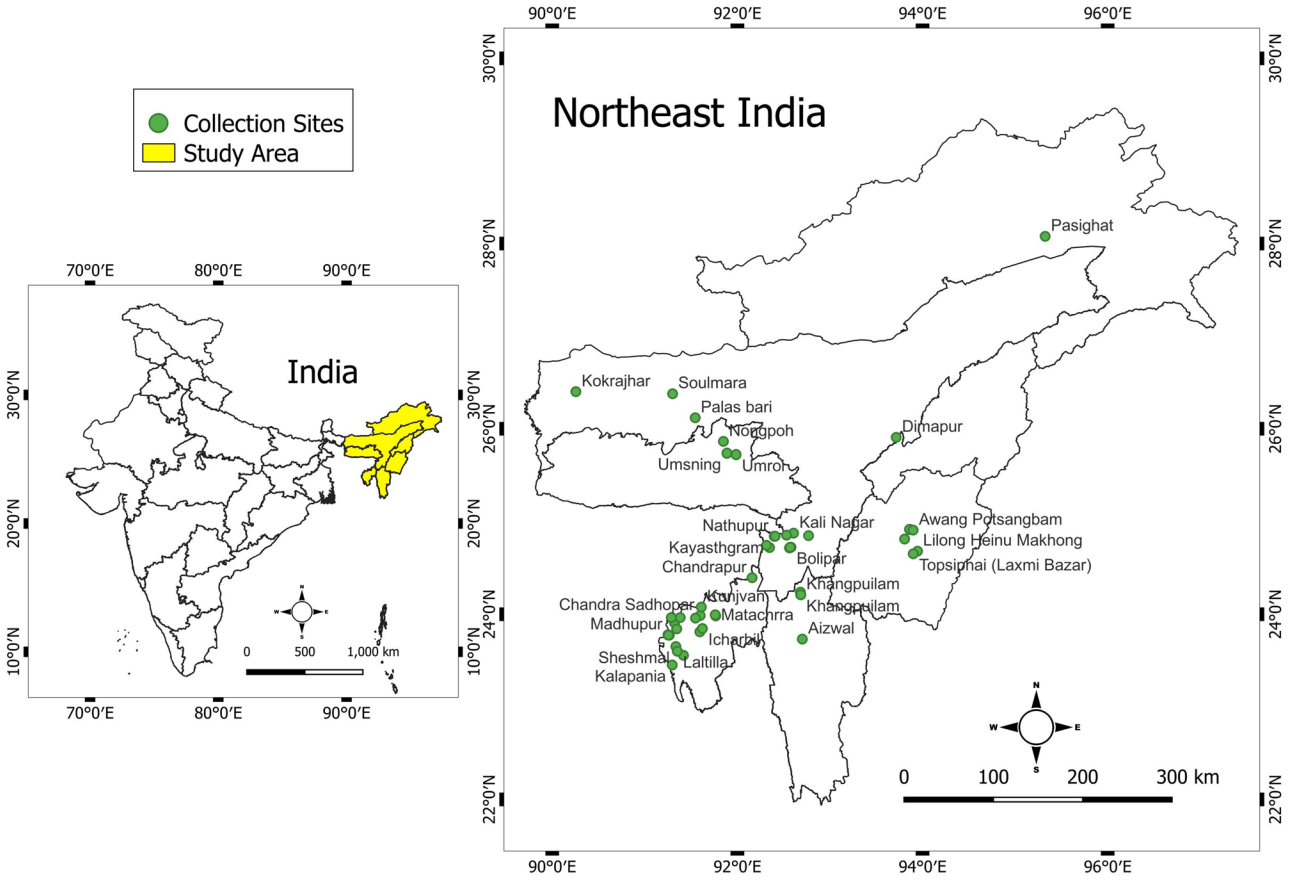

Fig. 1 Site of germplasm collections of the teasel gourd from northeastern states of India

et al. [33]. The quantification of ascorbic acid was carried out through the direct colourimetric method by spectrophotometrically following the procedure described by Sadasivam and Manickam [34].

Statistical analysis for quantitative traits

The mean of all the replicates were used to calculate the analysis of variance [35] using R software. The genetic parameters of including phenotypic and genotypic variances were estimated by following the methodology as described by Burton and de Vane [36], heritability by Hanson et al. [37], and genetic advance by Johnson et al. [38]. The AMMI (Additive Main Effects and Multiplicative Interaction)-based stability parameters (ASTABs) of the fruit traits from the observations of four years (2018-22) were measured, including AMMI stability value (ASV) and AMMI Stability Index (ASI) using the "METAN: Multi Environment Trials Analysis" package (v. 1.16.0) in R version 4.2.1[39, 40].

Molecular characterisation

DNA extraction, electrophoresis, simple sequence repeats (SSR) markers

The genomic DNA was extracted from fresh and young leaves of newly sprouted shoots (one-month-old plants) using the modified cetyltrimethylammonium bromide (CTAB) method [41]. Quantification of the DNA was measured directly using a NanodropTM 1000 Spectrophotometer (Thermo Scientific, USA). Thirty-five SSR primers reported earlier by Guo et al. [42] and Saxena et al. [15] in *Momordica charantia* L. and 10 SSR primers reported by Machida-Hirano et al. [43] in *Sechium edule* were chosen. Forty-three SSR primers have applied transferability to the teasel gourd genotypes. The genomic DNA (10 ng) from each genotype was amplified in a reaction mixture of 20 µl that contained 1 x Taq buffer, two mM $MgCl_2$, 0.25 mM dNTPs, 0.25µM of each primer, and 0.5 U of Taq polymerase (Thermo Scientific, India), using a Veriti PCR machine (Applied Biosystem, USA). The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation (94 °C for 45 s), annealing (55 °C for 45 s) and extension (72 °C for 45 s) followed by final extension at 72 °C for 5 min. The amplification products were electrophoresed on a 3%

Super Fine Resolution (SFR) agarose gel in 1 x TAE (Tris-acetate EDTA) buffer at 60 volts constant power supply. Ethidium bromide (EtBr) was added to the gel for staining in order to visualize the DNA amplicons and ChemidocTM (BioRad, California, USA) was then used for further documentation.

Statistical analysis

The PCR products were used to score the alleles. A 50-bp DNA ladder (Thermo Scientific, India) was used to determine the molecular weights of polymorphic bands, and the gel's migration distance was used to establish the bands' homology. The significant allele frequency and polymorphism information content (PIC) for each locus were determined using Power Marker software. The software GenAlEx v.6.1 determines the pairwise Nei's genetic distance and private alleles. Using MEGA 7 software, a neighbor-joining dendrogram (cluster analysis) based on Nei's genetic distance for genic-SSR was produced. STRUCTURE 2.3.4 software [44] was used to analyze population structure. The optimal K value was ascertained by importing the results of the STRUCTURE 2.3.4 software into the Structure Harvester online program at http://taylor0.biology.ucla.edu/structureHarvester [45]. The XLSTAT program was used to analyze the principal coordinate analysis (PCoA) based on allele frequencies.

Induction of monocliny and response of pollen to different nutrient medium

Response of $AgNO_3$ to induce monocliny

To induce hermaphroditism, four different treatments, namely T_0: Control (water), T_1: $AgNO_3$ (200 mg l^{-1}), T_2: $AgNO_3$ (300 mg l^{-1}), T_3: $AgNO_3$ (400 mg l^{-1}), and T_4: $AgNO_3$ (500 mg l^{-1}) were applied to the fully developed one-month-old teasel gourd females propagated by sprouted roots in three replications comprised of three plants each. The observations were recorded for the PV: pollen viability (%), FS: fruit set (%), FW: fruit weight (g), FL: fruit length (cm), FD: fruit diameter (cm), and DOE: duration of the efficacy (days).

Response of the nutrients medium for pollen germination

The pollen germination percentage was counted from the flowers of the induced monoclinous flowers and male flowers under the microscope (Leica Microsystems). The pollen germination was assessed from the freshly opened flowers placed in the cavity slides, containing 0.8% agar with different combinations of sucrose, boric acid, and calcium nitrate at room temperature (22–25 °C). The total number of counts for each treatment ranged from 202 to 232. Four treatment were applied as the following: T_0: control, T_1: sucrose (10%), boric acid (15 mg l^{-1}) and calcium nitrate (25 mg/L), T_2: sucrose (10%), boric acid (20 mg l^{-1}) and calcium nitrate (25 mg l^{-1}), T_3: sucrose (15%), boric acid (25 mg l^{-1}) and calcium nitrate (25 mg l^{-1}), and T_4: sucrose (20%), boric acid (25 mg l^{-1}) and calcium nitrate (25 mg l^{-1}) to select the best nutrient mediums for germinating the pollen of the male and induced monoclinous flowers of the teasel gourd as well as the male flowers of the bitter gourd for the comparative study. Observation for the pollen germination percentage (male) was recorded at 8, 24, and 48 h after the plating.

Data analysis

To ascertain the statistical significance of the treatment effect, the mean data was subjected to an analysis of variance using R software. Duncan's Multiple Range Test (DMRT) was performed to complete the multiple means comparison by comparing the least squares means of the respective treatment combinations at 0.05 probability significant levels.

Results

Genetic variability for growth, yield, and quality traits

Out of 70 genotypes of the teasel gourd (including eight males), 62 female genotypes were evaluated for the 24 growth, yield, and quality traits. The geographical information/passport data of the collection sites were recorded during the exploration, indicating wider adoptability of the crops, and were distributed from − 11 to 887 m above mean sea level in different growing pockets of the Northeastern states of India (Supplementary Table 1). Wide variability was observed for the flower colours, from cream-yellow to orange. The flower has five petals with 3 (Fig. 2a & b) or 5 (Fig. 2c) major black spots on the base of the petals, or three primary along with two minor black spots (Fig. 2d) on the petals. The leaves were cordate in shape with a moderate to highly serrated leaf margin (Fig. 2e-g). Likewise, fruits with different shapes and sizes have soft or hard spines (Fig. 2h). Significantly, wide variability has been observed for all the quantitative traits (Table 1), such as FFN for female flowers from 6.33 to 17.0 cm, OL at flowering 0.58–1.23 cm, SL 0.37–1.10 cm, SW 0.09–0.50 cm, PL 2.23–7.07 cm, PW 1.53–4.93 cm, FW 22.80–129.33 g, FL 4.76–11.23 cm, FD 3.03–6.13 cm, STL 0.25–1.30 cm, STD 0.20–0.78 cm, PDL 8.90–25.07 cm, PDD 0.14–0.40 cm, NFPP 4.0–25.33 cm, and YPP 0.15–2.66 kg/plant. Likewise, variation was also noted in seed and leaf characteristics such as HSW 12.60–36.09 g, SDL 0.91–1.30 cm, SDW 0.63–0.93 cm, SDT 0.44–0.66 cm, LFL 6.70–13.57 cm, and LFW 6.67–12.77 cm. The quality traits such as TSS, RS, and VC content have also shown wider variability, ranging from 2.47 to 5.43, 2.99–7.39, and 44.80–79.33, respectively. For all the traits (Table 1), the phenotypic coefficient of variance (PCV) was higher than the genotypic coefficient of variance (GCV). Except for the first flowering nodes, all the traits have shown high heritability (> 60%), and except for

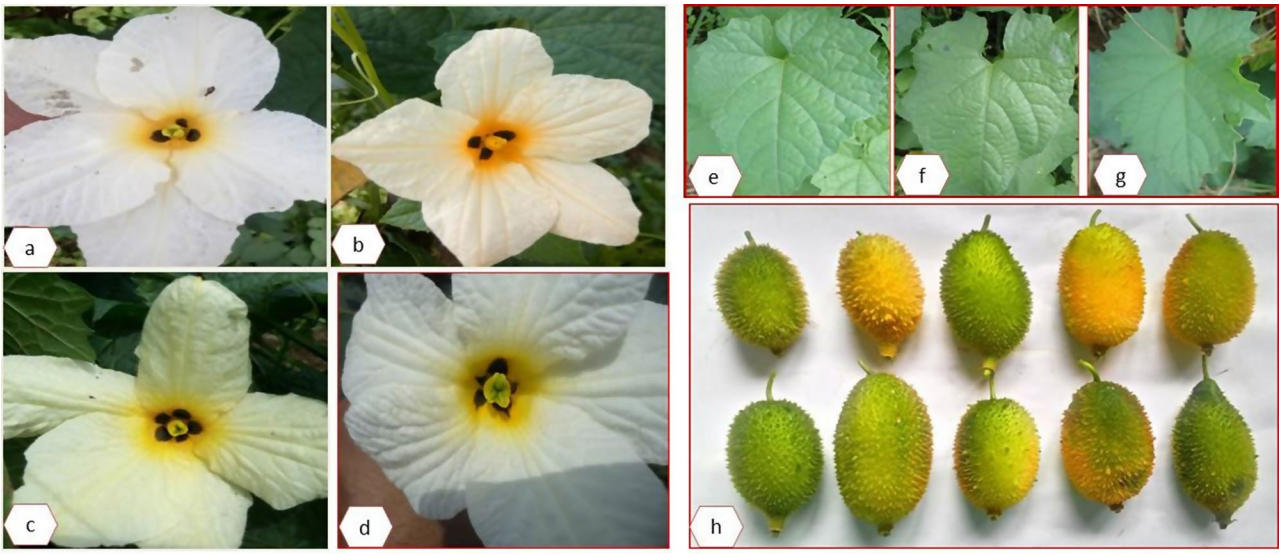

Fig. 2 Variability in teasel gourd for flower, leaves and fruits

Table 1 Analysis of genetic parameters for growth, yield and quality traits in teasel gourd

Traits	Mean	Range	CD	GCV (%)	PCV (%)	h2	GA	GAM
FFN	9.93	6.33-17.0	2.65	15.21	22.45	45.86	2.11	21.21
OL	0.93	0.58–1.23	0.11	12.71	14.65	75.27	0.21	22.72
SL	0.68	0.37–1.10	0.11	22.26	24.35	83.58	0.29	41.93
SW	0.15	0.09–0.50	0.04	70.41	72.22	95.04	0.22	141.42
PL	4.94	2.23–7.07	0.31	19.53	19.92	96.13	1.95	39.44
PW	3.61	1.53–4.93	0.25	20.63	21.05	96.00	1.50	41.63
FW	66.44	22.80-129.33	10.08	29.48	30.94	90.80	38.45	57.87
FL	7.71	4.76–11.23	0.74	17.33	18.31	89.54	2.60	33.78
FD	4.54	3.03–6.13	0.52	10.55	12.68	69.22	0.82	18.08
STL	0.78	0.25–1.30	0.10	28.80	29.82	93.28	0.44	57.30
STD	0.41	0.20–0.78	0.06	24.38	26.16	86.84	0.19	46.79
PDL	15.79	8.90-25.07	1.93	20.91	22.24	88.46	6.40	40.52
PDD	0.23	0.14–0.40	0.02	17.88	18.91	89.47	0.08	34.83
NFPP	12.50	4.0-25.33	2.36	46.12	47.58	93.96	11.51	92.09
YPP	0.85	0.15–2.66	0.22	58.42	60.54	93.11	0.99	116.12
HSW	22.51	12.60-36.09	3.02	17.32	19.20	81.36	7.24	32.18
SDL	1.08	0.91–1.30	0.07	7.29	8.44	74.70	0.14	12.98
SDW	0.79	0.63–0.93	0.05	7.73	8.62	80.43	0.11	14.29
SDT	0.54	0.44–0.66	0.03	49.00	55.56	77.78	0.48	89.14
LFL	9.88	6.70-13.57	0.86	13.48	14.53	86.11	2.55	25.77
LFW	9.36	6.67–12.77	0.91	13.49	14.76	83.53	2.38	25.39
TSS	3.68	2.47–5.43	0.27	14.71	15.40	91.17	1.07	28.93
RS	4.52	2.99–7.39	0.19	19.74	19.90	98.33	1.82	40.32
VC	60.12	44.80-79.33	3.60	16.10	16.52	94.96	19.43	32.31

Where: CD= Critical difference at 5%, GCV= Genotypic coefficient of variance, PCV= Phenotypic coefficient of variance, h^2= Heritability (Broad Sense), GA= Genetic advance, GAM= Genetic advance as a percentage of the mean

seed length and width, all the traits have shown higher genetic advance as a percentage of the mean (> 20%). The genetic advances of these two seed traits were moderate (10.0–20.0%).

Principal component analysis (PCA)

The results of the PCA analysis of twenty-four traits presented in Table 2 have shown a wide dimension with a distribution over 8 PCs having Eigen Values (EV) of over 1.0 and a cumulative percentage of the variance of 71.53. Moreover, the first three PCs accounted for 40.16% of the

Table 2 Principal component analysis for growth, yield and quality traits in teasel gourd

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
FFN	-0.27	-0.07	0.07	-0.11	0.03	0.40	-0.58	0.12
OL	0.50	-0.30	0.32	-0.16	0.01	-0.09	-0.02	0.30
SL	0.19	0.42	0.23	-0.07	0.32	0.32	0.15	0.17
SW	-0.13	0.72	-0.22	-0.08	0.06	0.34	-0.09	-0.06
PL	0.31	-0.66	-0.16	0.24	0.37	0.13	-0.33	-0.02
PW	0.28	-0.79	-0.01	0.23	0.26	0.20	-0.17	-0.06
FW	0.81	-0.04	0.13	-0.14	-0.39	-0.09	0.14	0.14
FL	0.45	-0.39	-0.09	0.02	-0.54	0.12	0.13	-0.17
FD	0.70	0.05	0.32	0.04	-0.14	-0.24	-0.14	0.14
STL	-0.01	0.06	0.55	0.08	0.01	0.20	0.13	-0.32
STD	0.05	0.24	0.61	-0.45	-0.01	0.04	-0.04	0.16
PDL	0.20	0.06	0.38	0.07	0.44	-0.25	-0.10	-0.14
PDD	0.32	-0.09	0.12	0.37	0.29	-0.02	0.35	-0.28
NFPP	0.50	0.33	-0.55	-0.05	0.35	-0.03	-0.08	-0.19
YPP	0.79	0.2	-0.39	-0.16	0.08	-0.08	0.02	-0.05
HSW	0.16	0.48	0.10	0.57	-0.06	-0.18	-0.25	0.01
SDL	0.45	0.33	0.25	0.40	0.04	-0.12	-0.15	0.38
SDW	0.28	0.31	0.10	0.69	-0.10	0.01	-0.11	-0.03
SDT	0.28	0.06	0.09	-0.03	-0.61	0.12	-0.04	-0.28
LFL	0.78	0.00	-0.12	-0.12	0.16	0.25	0.08	0.08
LFW	0.73	0.06	-0.04	-0.15	0.01	0.32	0.08	-0.01
TSS	0.34	0.09	0.06	-0.57	0.19	0.01	-0.30	-0.39
RS	-0.12	-0.12	0.55	0.16	-0.01	0.32	0.21	0.09
VC	-0.01	-0.06	0.53	-0.23	0.28	-0.20	-0.18	-0.38
Eigenvalue	4.59	2.71	2.34	1.96	1.72	1.52	1.20	1.11
Percentage of variance	19.13	11.27	9.77	8.17	7.15	6.37	5.03	4.65
Cumulative percentage of variance	19.13	30.4	40.16	48.34	55.48	61.85	66.88	71.53

total variability. PC1 contributed 19.13% of the total variation with loadings > 0.4 and attributed positively to economically important traits of FW, YPP, LL, LW, FD, OL, and NFF. PC2, which accounted for 11.27% of the overall variability, linked to SL and seed characteristics of HSW, SDL, and SDW. The important contributing traits of the PC3, which accounted for 9.77% of the overall variability, were OL, STL, STD, FD, PDL, RS, and VC. PC4 was primarily attributed to seed characteristics and contributed 8.17% of the total variability. The PCA biplot, as shown in Fig. 3, also clearly distinguished the genotypes for the traits over the varied components. PC1 distinguished the genotypes for the traits SDW, STL, FFN, VC, and RS from other characteristics. Likewise, PC2 distinguished the genotypes for the seed traits.

Genetic diversity based on quantitative traits

The cluster analysis based on 24 different quantitative traits (Supplementary Table 2), as presented in Fig. 4, have demonstrated that the presence of wide diversity among the 62 female genotypes of teasel gourds collected from various regions in the Northeastern states of India was categorized into three major clusters. Cluster I comprised 25 genotypes, which were further divided into two sub-groups, and among them, the high yielding genotypes ASTGC-11(2.66 kg/plant) and ASTGC-12 (1.24 kg /plant) collected from the Barak Valley, Assam, were found to be the most diverse among the rest of the genotypes. Similarly, cluster II was further grouped into three sub-groups and sub-group I comprised 11 genotypes. At the same time, sub-group II had 21 genotypes, and sub-group III had the single genotype MNTGC-1 and was found to be the most diverse in cluster II. Moreover, Cluster-III was grouped by five genotypes from Manipur, Meghalaya, Assam, and Tripura. Among the genotypes, the utmost genetic distance was found to exist between ARTGC-2 from Arunachal Pradesh and ASTGC-12 (Assam), followed by ARTGC-2 to ASTGC-11 (Assam), and TRTGC-17 (Tripura) to ASTGC-12 (Assam). Moreover, the lowest genetic distance was between the genotypes ASTGC014 and MLTGC-10, followed by TRTGC-14 and TRTGC-1, and TRTGC-13 and TRTGC-5. The genotypes of different geographical origins were distributed in other clusters. The genotypes from Manipur were grouped in clusters II and III, and the Mizoram genotypes were in clusters I and II. Genotypes of cluster-1 were found to be superior for the FW, FL, FD, STL, STD, TSS, RS, and VC, with mean cluster values of 82.47, 8.23, 4.86, 0.78, 0.44, 3.92, 4.53, and 65.07, respectively. The genotypes of cluster III were superior

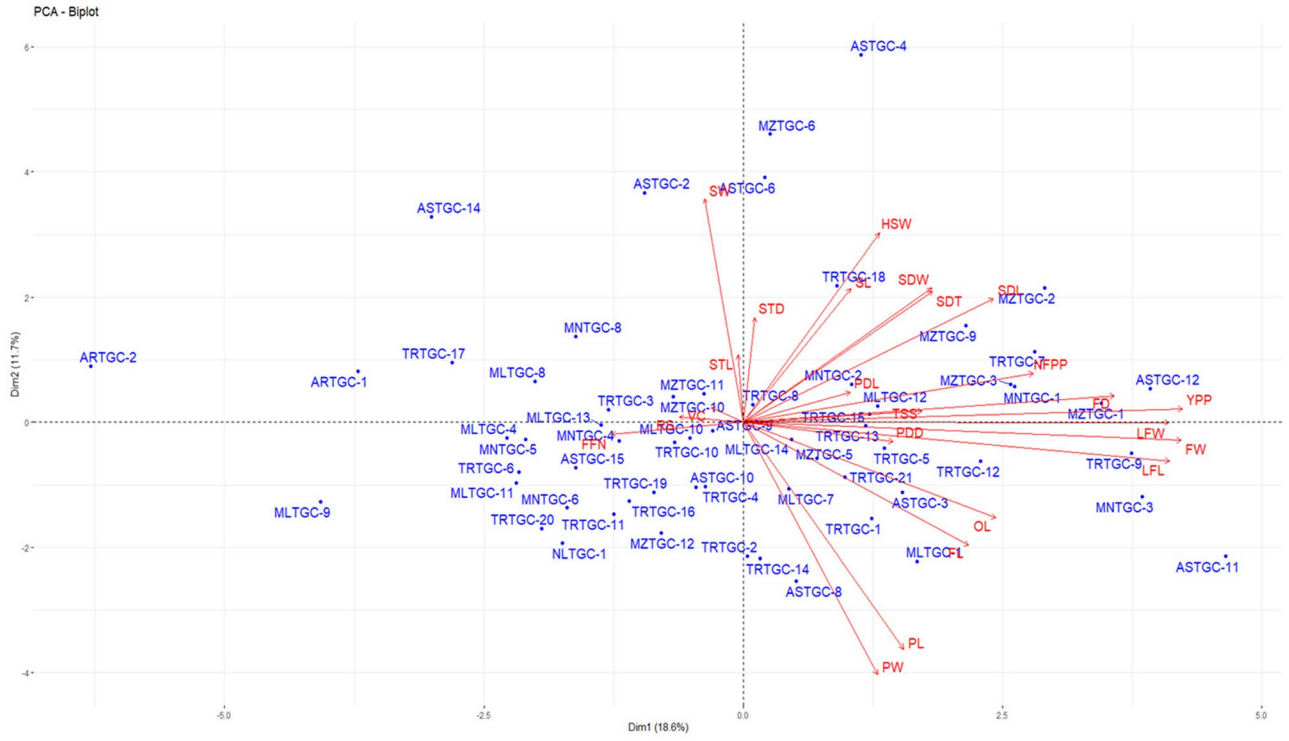

Fig. 3 Principal component analysis (PCA) biplot quantitative traits in teasel gourd

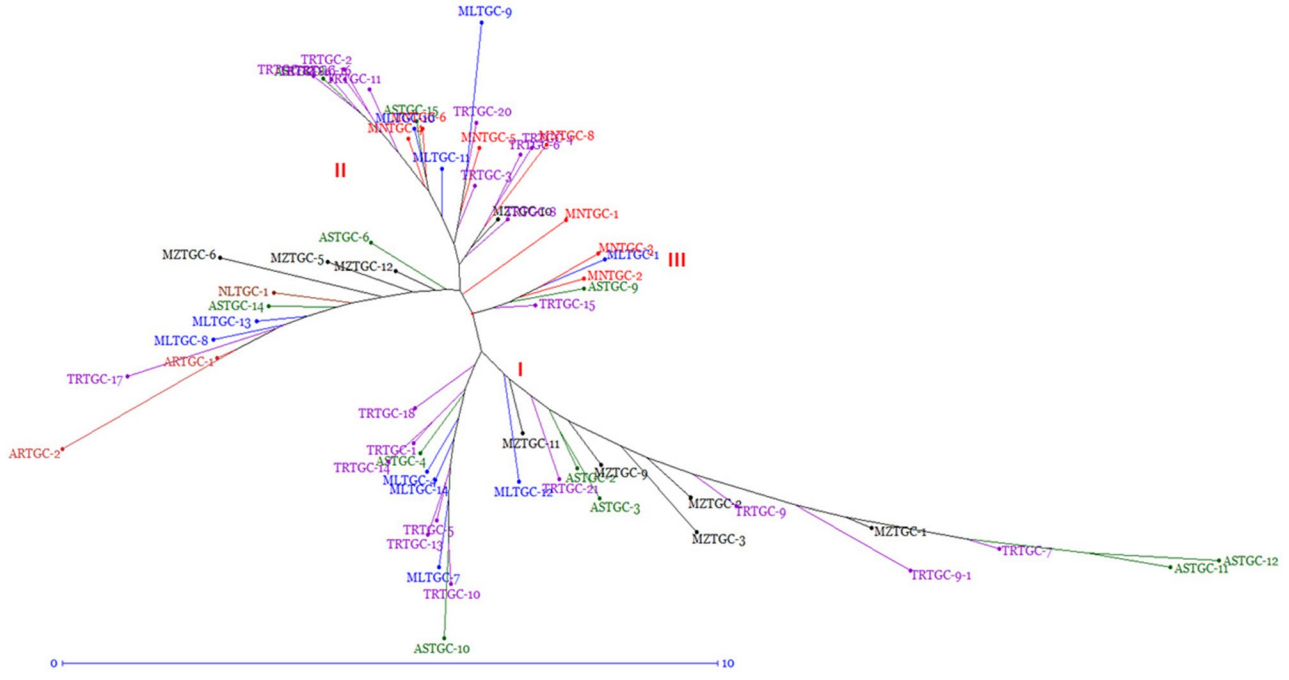

Fig. 4 Phylogenetic relationship among the female genotypes (62) of the teasel gourd based on quantitative traits

for NFPP, YPP, HSW, and SDW, with cluster mean values of 22.2, 1.426, 24.136, and 0.812, respectively.

Molecular diversity

Allele diversity

The analyses of molecular markers have shown wide allelic variation among the genotypes of the teasel gourd (Supplementary Fig. 1). Out of 45 SSR markers used in the study, 43 have shown amplification and 40 were polymorphic. The total numbers of polymorphic alleles were 100 and with a mean of 2.50 alleles per marker (Table 3). Three markers, namely, MCSSR-14, MCSSR-18, and Sed-11, were monomorphic and maximum number of alleles 4.0 was observed in the markers A-4, C-24, McSSR-6, McSSR-56, and Sed-01. The number of effective alleles also ranged from 1.0 to 3.63 (McSSR-6), with a mean of 1.97. The allele frequency ranged from 0.40 (McSSR-86) to 1.0 (monomorphic markers). Out of 43 markers, eight have shown heterozygosity in the genotypes of teasel gourd. Among these markers, Sed-08 from *Sechium edule* was monomorphic and has shown heterozygosity for all 70 genotypes, including males. Comparatively, the population's expected heterozygosity of 0.435 was higher than the observed heterozygosity of 0.123. The average PIC was also moderate (0.384) and ranged from 0.114 (McSSR-20) to 0.676 (McSSR-6). High gene diversity was also observed for the marker McSSR-6, similar to the PIC value. The markers with high PIC value (> 0.5) were high for their corresponding Shannon information index value (> 1.0). Among the markers, the highest PIC and Shannon information index was observed for McSSR-6 (0.676 and 1.335), McSSR-86 (0.586 and 1.091), Sed-01 (0.586 and 1.13), C-30 (0.519 and 1.062), Sed-05 (0.580 and 1.083), and McSSR-3. Out of 43 SSR markers, one marker, A-4 (325 bp), was specific to male accessions, while 7 markers, namely C-30 (225 bp), McSSR-3 (165 bp), McSSR-5 (265 bp), McSSR-16 (145 bp), McSSR-28 (200 bp), Sed-06 (290 bp), and Sed-09 (410 bp), were specific to female accessions.

Population diversity

According to molecular variance analysis, the presence of significant variation has been observed among the population (0.36%), within (24.0%), and between (76.0%) the male and female individuals of teasel gourd (Table 4). Significant variations have also been observed in the populations based on geographical origin. The maximum variations have been observed within the population (82.87%), followed by among the population (17.13%) of different geographical origins (states). Comparatively, the results of group-based observed and expected heterozygosity, Shannon information index, and the number of effective alleles have shown the presence of greater diversity in the female population over the male population (Table 5).

Nei's genetic distance-based cluster analysis, as presented in Fig. 5, has also exhibited wide diversity in the male and female genotypes of the teasel gourd. All genotypes were grouped into four major clusters. The males (red circle) and females were distributed to all the clusters without any differentiation for the geographical locations. Similarly, principal coordinate analysis (PCoA) has also shown the distribution of the genotypes across the axis without any differentiation for males and females, as well as the geographical location of the genotypes. With 18.45% of the variation specified by the first coordinate and 11.58% by the second, the first three PCoA have accounted for 40.69% of the overall variation (Fig. 6).

Genetic structure and interrelationship

The optimum cluster number (K = 2) was determined at maximal ΔK (482.80) using the STRUCTURE software, as per the procedure described by Evanno et al. [46] (Fig. 7a). Both males and females were differentiated into two major groups (Fig. 7b), with three categories of population: pure (2) and mixed (1). The proportion of genotypes with admixture was 2 out of 8 male and 23 out of 62 female genotypes (at the 95% significance level). Regarding geographical origin, the admixture in males was observed in MLTGM-6 and TRTGM-1 a collection from Meghalaya and Tripura, respectively. The proportion of admixture in female was significantly higher in collections of Meghalaya (70%) followed by Mizoram (44.44%) and Tripura (16.00%) (Fig. 7b). The genotypes from Assam (Barak Valley), Mizoram, and Manipur were found close.

Stability analysis for fruit traits

AMMI analysis of variance

The results of the analysis of variance indicate that the teasel gourd's fruit attributes are significantly affected ($p < 0.01$) by genotypes (fixed), years /environments (random), and genotypes by environment interaction (Table 6). Among the factors, genotype explained significantly, i.e., 69.49, 45.98, and 73.59% of the total variation for FL, FD, and FW, respectively. The first three G × E interaction principal components (IPCA) explain 100% of the variation and were found significant for all traits. The IPCA1 explained 46.40, 55.30, and 59.6%; the IPCA2 explained 38.20, 26.20, and 24.8%; and the IPCA3 explained 15.50, 18.60, and 15.70% of the G × E interaction for FL, FD, and FW, respectively.

AMMI stability analysis

In the AMMI1 biplot, the main effects (genotype mean and environment mean) are plotted against IPCA1 scores for both genotypes and environments. For fruit

Table 3 Summary statistics of microsatellite markers used for characterization of teasel gourd germplasm

Marker	Forward Primer (5′ → 3′)	Reverse Primer (5′ → 3′)	Repeat Motif	Na	Ne	MAF	Ho	He	GD	PIC	I*
A-2	GCGATGGTTGAGTGTTCC	TTCTTGTTGCCCTTGCTG	$(TA)_{9}\ldots(AC)_{15}$	4.00	2.209	0.586	-	0.730	0.544	0.465	0.934
A-47	TGGAATGGCAACTACACG	GGGGAGGCTGAAAGACTA	$(GT)_{15}\ldots(TA)_{5}$	2.00	1.516	0.786	-	0.150	0.337	0.280	0.524
C-4	TACTCTCCCTGATTCTTTATTT	TCACCAATCGCCAAATCT	$(GA)_{32}$	2.00	1.974	0.564	0.769	0.490	0.492	0.371	0.686
C-11	GAGATTGATGAGTCCTGAGTAA	AGAGAAAGAGTGAAC-CAAACAA	$(AG)_{17}$	3.00	1.647	0.757	0.692	0.470	0.368	0.300	0.630
C-9	AGTGGCGATTTTGGATAAGT	ATGAGTCCTGAGTAATTGCA	$(TC)_{19}$	2.00	1.931	0.586	-	0.270	0.485	0.368	0.675
C-17	GAGATTGATGAGTCCTGAGTAA	TGAACTGGCTTTACATC-TACCC	$(AG)_{26}\ldots(AG)_{9}$	2.00	1.664	0.729	-	0.520	0.396	0.317	0.589
C-1	GAGATTGATGAGTCCTGAGTAA	AAAAACTACATCTCCT-GTCTGA	$(CT)_{20}$	2.00	1.950	0.586	0.615	0.520	0.485	0.368	0.680
C-7	AAAACTTGTCCTCCACCG	TGTTGAGAAGATA-AAAGGATGA	$(GA)_{14}$	3.00	2.037	0.536	-	0.520	0.510	0.394	0.754
C-24	TGGCTCAGTATCGCAAGTAT	GAGGAGGAAGTTT-GACCTATGA	$(CT)_{19}$	2.00	1.800	0.657	-	0.520	0.451	0.349	0.637
C-30	CAGGGGCGATTAGATTATTC	AGGAGAAGGCTGTGTATGAA	$(CT)_{13}\ldots(CT)_{19}$	4.00	2.324	0.614	-	0.570	0.564	0.519	1.062
McSSR-3	TTTTGTCAATTTTCCCGACG	TTTCATCTTCCTCTC-GATCTCC	$(AAAGG)_{2}(GGAGA)_{2}G(AGAGGA)_{2}$	3.00	2.632	0.500	-	0.490	0.617	0.543	1.028
McSSR-5	CTTTAACTCACCTTCCACACCC	ACGATATGATCGAATGTC-CACC	$(CTT)_{3}GA(TCT)_{4}TTTTC(TCTT)_{2}$	2.00	1.553	0.757	-	0.440	0.368	0.300	0.542
McSSR-6	CGTGATTTTGTTTCGCCACC	TAAAACCGAAACCGAAACCC	$(AG)_{4}C(GGAG)_{2}(AAG)_{3}(AG)_{6}$	4.00	3.632	0.357	-	0.650	0.726	0.676	1.335
McSSR-13	GTTCGGGATCTTCTTGCTCG	TCCCTTCTCCCCATCTCTCC	$(GA)_{6}$	2.00	1.441	0.814	-	0.440	0.302	0.257	0.484
McSSR-14	TTGCATGCTTTTTGGTAGAGC	GACTCATCTACCGAAT-CAACGG	$(TA)_{6}$	-	-	-	-	-	-	-	-
McSSR-16	GGCTTCCTTCAGTGAGTGCG	GTCTGTCGATGCGTCTTCGG	$(TTC)_{5}$	3.00	1.996	0.671	-	0.280	0.494	0.443	0.865
McSSR-23	AGGTGGCCCTCTCTCAATCT	TATGTCGGCAGTCTCCCTCT	$(GCAT)_{2}$, $(AATG)_{2}$	2.00	1.734	0.700	-	0.370	0.420	0.332	0.615
McSSR-26	TCCATTTTCTTTCGCAATCC	TGTTATTGGCTCCCTCTGCT	$(GCG)_{4}$, $ACAC(TGAG)_{2}$, $(TGAA)_{2}$	2.00	1.700	0.714	-	0.370	0.408	0.325	0.602
McSSR-20	GGAATTCAGGTGAACCTGACG	CCAGGAGGAAGAG-GAACTGC	$(TCT)_{9}$	2.00	1.189	0.914	0.231	0.210	0.157	0.144	0.295
McSSR-18	TAAAGAATCGGCCAGTTCGG	GGGGTTAGAGAAAAT-GAGAGGC	$(AT)_{8}$	-	-	-	-	-	-	-	-
McSSR-27	ATTTCCATTTCGCGATTCAG	GCCTTGTTTTCCGAAAGA-GAT	$(TCTCGA)_{2}$	2.00	1.830	0.657	-	0.270	0.451	0.349	0.646
McSSR-29	TGCCATTTCGGGTTAAGAAG	CTGCGGAAAAATAGCTCGAC	$(CT)_{5}$, $(GA)_{4}$, $(TTTTCAT)_{2}$	2.00	1.859	0.643	-	0.270	0.459	0.354	0.655
McSSR-32	CCGATCCTTGTTTACCAACC	TCTCGAGAAACAAGTGGGC-TA	$(TC)_{4}$	2.00	1.700	0.714	-	0.270	0.408	0.325	0.602
McSSR-34	ACGCCAACGATATACCACCT	CCCATGGTTTGAGGTCATTC	$(GC)_{4}$, $(CACGCG)_{2}$	2.00	1.990	0.543	-	0.440	0.496	0.373	0.691
McSSR-54	CCATCCATATCCCAATTCCA	TCATCA-CAAACCTCCCTTTTTC	$(ATCAT)_{2}$	2.00	1.800	0.671	-	0.520	0.441	0.344	0.637
McSSR-60	TAGTTGATGGCACGTTGCTC	GACACCCGACCTAGGAGTTG	$(AGCTTG)_{3}$	2.00	1.980	0.557	-	0.440	0.493	0.372	0.688
McSSR-22	CCATGACCGATGTAGCACTCC	TCGAACCAACCTAAACCAG	$(GGTTC)_{3}$	3.00	1.884	0.700	0.692	0.500	0.465	0.419	0.824
McSSR-47	TTGATTTTGAATCAGCGTTGT	ATTTTGCACAAGGCCTACCA	$(TA)_{4}$	2.00	1.768	0.671	-	0.370	0.441	0.344	0.626
McSSR-55	ATCCAACCAATAACCGGAAG	CTACCATTTTGGGGACGAGA	$(TC)_{4}$	3.00	1.842	0.686	-	0.670	0.466	0.405	0.782
McSSR-56	TGCCATACTCCCAGGAAAAG	CGGAGACCTGTGTTTTTGGT	$(CT)_{4}$	4.00	2.245	0.614	-	0.650	0.550	0.493	1.000

Table 3 (continued)

Marker	Forward Primer (5′ → 3′)	Reverse Primer (5′ → 3′)	Repeat Motif	Na	Ne	MAF	Ho	He	GD	PIC	I*
McSSR-68	CTTCTCTTTGCCCCTTACGA	CAGTGCCCCACAACTATGAA	$(AT)_4AG(AT)_2AG(AT)_4$, $(GAAA)_2(GGGC)_2$	2.00	1.886	0.629	-	0.520	0.467	0.358	0.663
McSSR-86	ACTCGTATGGGTGCCTTTTG	ATGTTGATTGGGCAGGAAGT	$(TCTA)_2$	3.00	2.952	0.400	-	0.630	0.660	0.586	1.091
McSSR-96	GCATGCTGAATTGTGTTGGT	GTGTAACAGCCCTCGACCAT	$(GTG)_3$, $(TTGG)_2$, $(AGTG)_2$	2.00	1.886	0.614	-	0.370	0.474	0.362	0.663
McSSR-100	TTAGGACCATTTGGGAGTGC	CCAAATCGTGCTCAAACTGA	$(AGGCGCC)_2$	2.00	2.000	0.514	-	0.370	0.500	0.375	0.693
Sed-01	CCCCGTTACCCTGACTCTCGAT	GGCTTGTTCAAGACTTC-GCAGC	$(CA)_8$	4.00	2.815	0.436	0.923	0.590	0.650	0.581	1.130
Sed-03	CGTATGGTCGAGGTGCGCATAA	AAGTCCAGAAATGTACACT-GCCACT	$(CA)_9$	2.00	1.931	0.586	-	0.490	0.485	0.368	0.675
Sed-04	GGCCCTTAGTTTGCTGATGGGT	TGGGACCCACGTGCTA-AAAGTG	$(CA)_2CT(CA)_2CC(CA)_3$	2.00	1.591	0.757	-	0.150	0.368	0.300	0.558
Sed-05	ACACACCTTAGAAAGAG-CAACCCC	GCTATGGCGCAAGTTGCT-GATG	$(CA)_2CGA(CA)_5$	3.00	2.908	0.414	-	0.570	0.654	0.580	1.083
Sed-06	AACCGCTGTTCTCTGCTCATCC	GGCTCAAGGTTGTTGTTG-GTGC	$(CA)_4TA(CA)_{16}$	2.00	1.830	0.657	-	0.270	0.451	0.349	0.646
Sed-07	AACCTGGGTCGTTACATGGTGC	ACCCTTGCCCTAGATGGTG-GAA	$(GA)_{34}$	3.00	1.996	0.571	-	0.370	0.502	0.389	0.743
Sed-08	AGCTCCTCCACCTCTACCTTTT-GC	ACTCTGGCGTATGGAAT-GACGC	$(GA)_3GC(GA)_5$	2.00	2.000	0.500	1.000	0.520	0.500	0.375	0.693
Sed-09	ACAGGCCACAGGGGAACAAAAT	CACGCCATCTCCGTC-CATCTTT	$(GA)_{11}$	3.00	1.268	0.886	-	0.120	0.209	0.198	0.436
Sed-11	TGGTCTGTTTGGCTCATCTCCA	TCGACCCCTAACCCTT-GAAGCT	$(GA)_{14}$	-	-	-	-	-	-	-	-
Mean				2.50	1.972	0.631	0.123	0.435	0.468	0.384	0.729

Where: Na = number of alleles; Ne = number of effective alleles; MAF=major allele frequency; Ho = observed heterozygosity; He = expected heterozygosity; GD= gene diversity; PIC = polymorphism information content and I = Shannon's Information Index for each marker

Table 4 Analysis of molecular variations (AMOVA) for male and female teasel gourd population

Source	Degree of freedom	Sum of square	Mean square	Est. Var.	Percentage of variation
Among populations	1	17.485	17.485	0.034	0.36%
Among Individuals	68	1123.323	16.519	7.135	76.0%
Within Individuals	70	157.500	2.250	2.250	24.0%
Total	139	1298.307		9.419	

length, genotypes TRTGC-17, TRTGC-4, TRTGC-8, and TRTGC-15 with average fruit length of 9.10, 8.74, 8.09 and 8.68 cm, respectively were found near the centre point and considered stable genotypes across the years (Fig. 8a). Likewise, the AMMI 2 biplot based on IPCA1 vs. IPCA2 explained the magnitude of interaction between genotypes and the environment (Fig. 8b). The genotypes TRTGC-3, ASTGC-3, MLTGC-5, and MLTGC-1 were found adjacent to the centre and stable for fruit length over the years, while genotypes TRTGC-14, MNTGC-4, MZTGC-1, TRTGC-1, and TRTGC-11 showed differences in mean fruit length over the years. Moreover, as per the AMMI stability value (ASV) and AMMI stability index (ASI), genotypes TRTGC-17, MLTGC-5, ASTGC-1, and TRTGC-13 were found to be most stable for fruit length (Table 7).

Similarly, for fruit diameter, the AMMI 1 biplot (Fig. 8c) identified the genotypes ASTGC-7, MLTGC-1, ASTGC-11, MZTGC-2, and MNTGC-4 as stable genotypes as they lay closer to the centre point of the biplot. In contrast, genotypes MNTGC-2 and ASTGC-3 were higher for average fruit diameter. The AMMI2 biplot

Table 5 Group based summary of diversity in teasel gourd based on SSR markers

Pop		Na	Ne	I	Ho	He	Percentage of polymorphic loci
Male	**Mean**	2.175	1.822	0.632	0.106	0.413	92.50%
	SE	0.094	0.075	0.041	0.042	0.026	
Female	**Mean**	2.325	1.945	0.708	0.114	0.461	100.00%
	SE	0.090	0.073	0.033	0.043	0.018	

Where: Na = No. of Different Alleles, Ne = No. of Effective Alleles, I = Shannon's Information Index, Ho = Observed Heterozygosity, He = Expected Heterozygosity

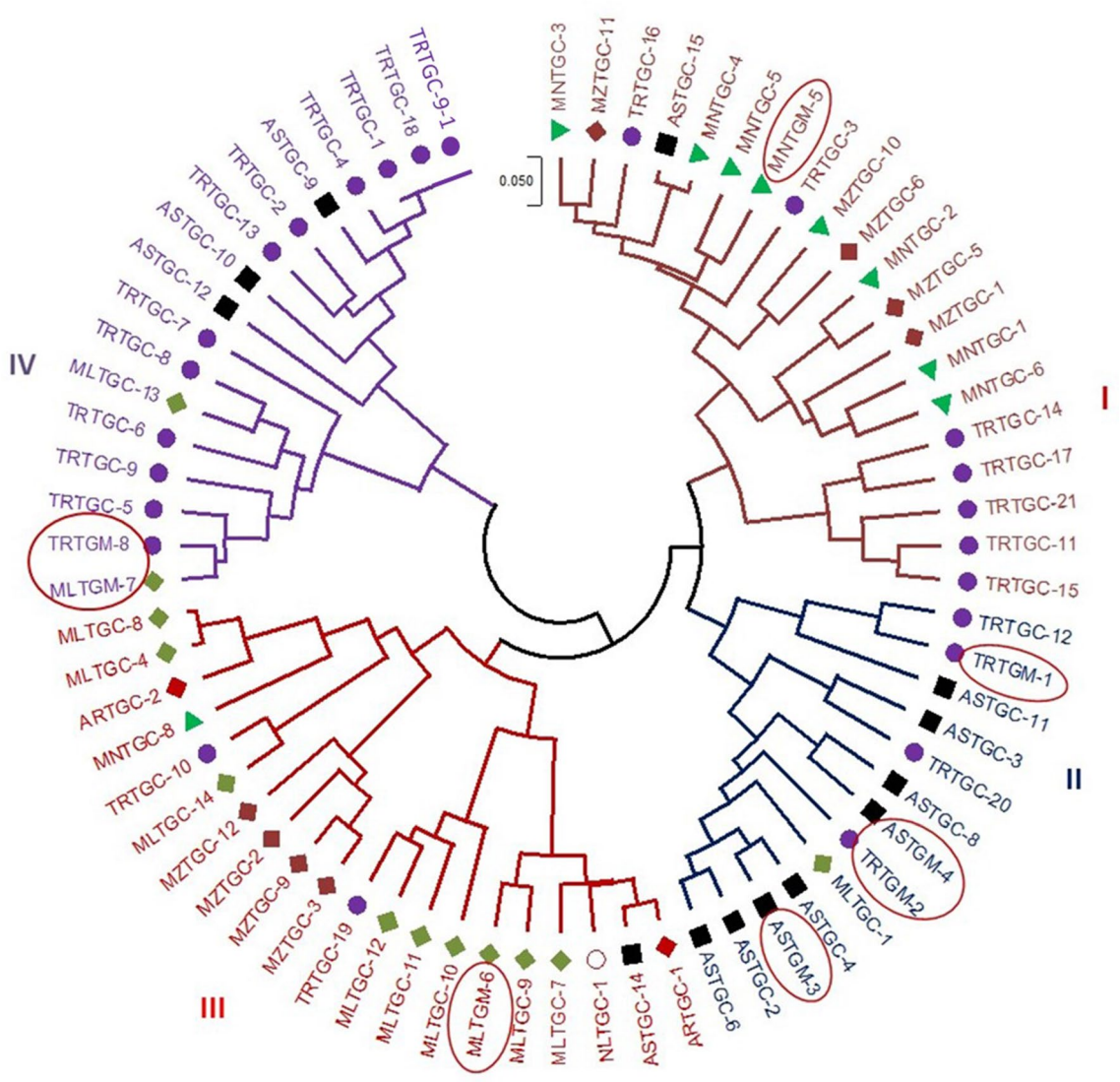

Fig. 5 Phylogenetic relationship among the male (red circle) and female genotypes of the teasel gourd based on 43 SSR markers

(Fig. 8d) differentiated the stable genotypes TRTGC-4, MZTGC-6, MNTGC-7, MNTGC-1, and ASTGC-4 from the unstable ones MLTGC-5, ASTGC-8, RCSG-17, and MLTGC-13, with differences in mean fruit diameter across the years. Moreover, the lowest ASV and ASI were observed for the genotypes TRTGC-4, ASTGC-7, MZTGC-6, and ASTGC-1 (Table 7).

Likewise, fruit weight was also affected by genotype, environment, and interactions. The AMMI 1 biplot (Fig. 8e) explained that genotypes ASTGC-7, TRTGC-4, ASTGC-11, and TRTGC-8 were in proximity to the centre of the biplot and stable, while genotypes MNTGC-1, MNTGC-2, MZTGC-1, and ASTGC-3 were high fruit weight average across the years. AMMI2 biplots (Fig. 8f) differentiated the stable genotypes (MLTGC-5, MLTGC-4, MNTGC-7, MZTGC-5, ASTGC-9, and MNTGC-4), which were least affected by GEI, while genotypes ASTGC-4, MZTGC-2, ASTGC-2, ASGC-8, and ASTGC-11 showed differences in mean fruit weight over the years. However, with the lowest ASV and ASI values, the most stable genotypes were MNTGC-2, MZTGC-5, and MNTGC-7 (Table 7).

Multi-trait stability index (MTSI) analysis

MTSI analysis was carried out to identify the stable genotypes for fruit traits across the environment/year. Based on MTSI, out of 36 genotypes, five accessions, viz., MNTGC-2, MNTGC-1, MNTGC-4, MZTGC-1, and ASTGC-3, were selected at 10% selection intensity for fruit traits. These genotypes were found superior for the fruit traits and crossed the cut-off point (red circle) as presented in Fig. 9. The findings of factor analysis, as presented in Table 8, have also explained that these fruit

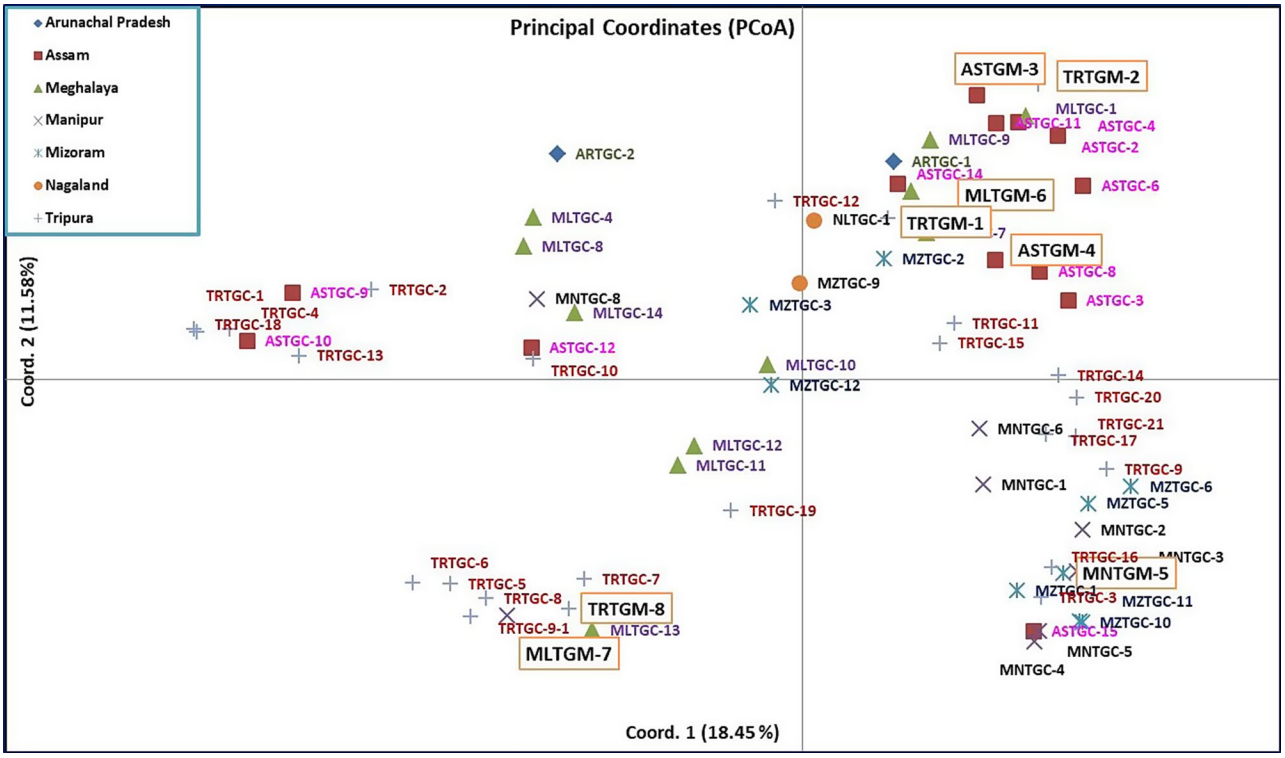

Fig. 6 Principal Coordinate Analysis among the male (bold) and female genotypes of the teasel gourd based on 43 SSR markers

traits are highly heritable and were found to be responsive to selection.

Induction of monocliny and response of pollen to different nutrient medium

Response of $AgNO_3$ to induced monocliny

The treatments have shown significant differences in the induction of hermaphroditism/monocliny in teasel gourd (Table 9). The crop is cross-pollinated by bumble bees (*Xylocapa* spp.), a chief pollinator. The male, female, and monoclinous flowers, monoclinous antherless (ALS) flowers, pollinator (bumble bees), and pollen viability and germination are presented in Fig. 10. It was observed that the female plants produced flowers during the initial 7–10 days after the treatments had shown the flowers with a poorly developed ovary that turned yellow and failed to fruit set in all the treatments. The PV in males was 95.45 ± 1.5%, while in the case of induced monoclinous flowers; it was 93.73 ± 1.5% without any differences among the treatments. The maximum FS (85.11%) was observed in control, while among the treatments, it ranges from 65.0% in T_4 (500 mg l^{-1}) to 74.33% in T_1 (200 mg l^{-1}), and it decreases with an increase in the concentration of $AgNO_3$. The FL, FD and FW were significantly reduced and were at maximum control over the treatments (Table 9). There was no significant difference between the treatments for FL, FD and FW of fruits. Further, the DOE of the treatments for inducing hermaphroditism ranges from 15.67 days (T_1) to 23.67 days (T_4), and it increases significantly with an increase in the concentration of $AgNO_3$. The treated plants at 500 mg l^{-1} $AgNO_3$ again turned into female plants and produced monoclinous antherless and natural female flowers 25 days after treatments (Fig. 10).

Response of nutrient media to pollen germination in male teasel gourd

The pollen germination from male teasel gourd has shown a significant response to the different growing media composition (Fig. 11). The wide variations were observed for the treatments, and it ranged from 17% in Control (T_0) to 77.8% (T_3) at 12 h after the incubation. Among the treatments, T_3: sucrose (15%), boric acid (25 mg l^{-1}) and calcium nitrate (25 mg l^{-1}) was found to be the most suitable medium with higher pollen germination, followed by T_4 at 12, 24, and 48 h. after incubation.

Comparative response of the nutrient media on pollen germination of male and induced monoclinous teasel gourd and male bitter gourd flowers

The pollens from the different *Momordica* spp., as well as induced monoclinous flowers, were highly responsive for germination to the selected growing nutrient media (Fig. 12). For male and induced monoclinous pollen of teasel gourd treatments T_3: sucrose (15%), boric acid (25 mg l^{-1}), and calcium nitrate (25 mg l^{-1}) were

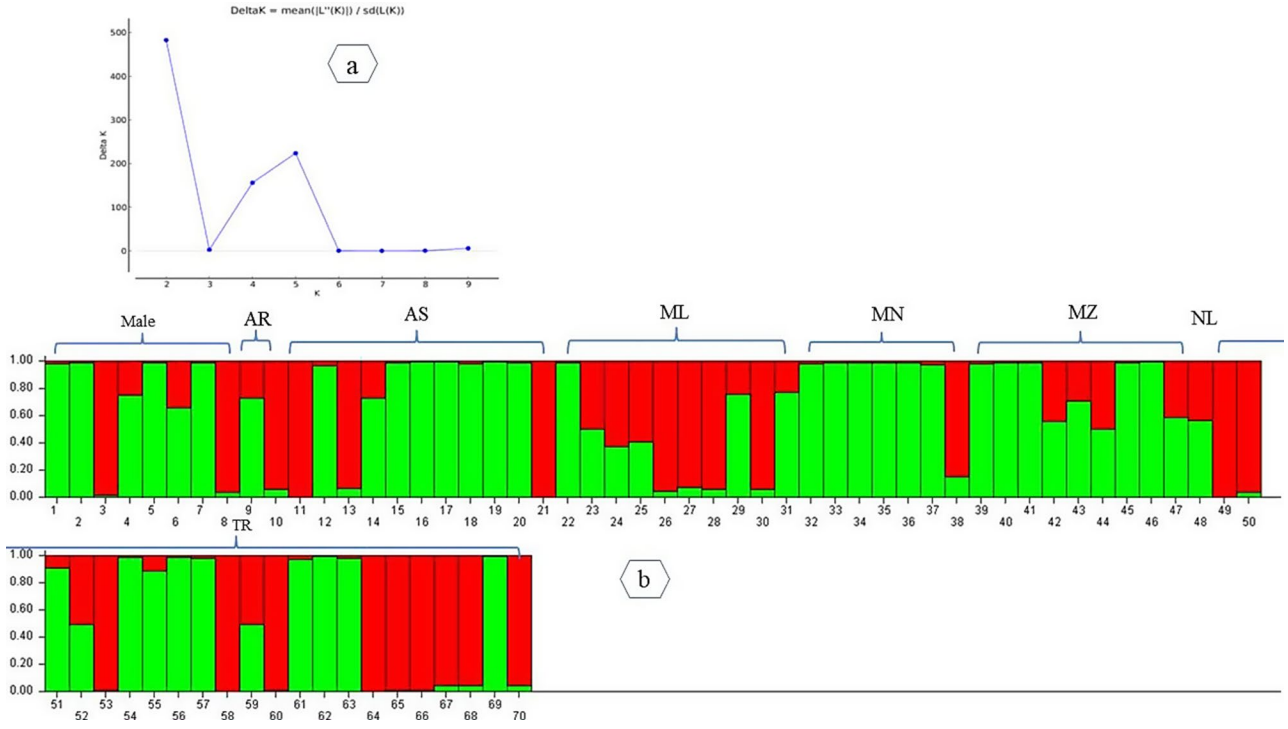

Fig. 7 Population structure of the accessions of teasel gourd based on SSRs. (**A**) ΔK graph, (**B**) Population structure at ΔK = 2. **Order of the genotypes**: **Male –** 1. ASTGM-3, 2.ASTGM-4, 3. MLTGM-7, 4. MLTGM-6, 5. MNTGM-5, 6. TRTGM-1, 7.TRTGM-2, 8.TRTGM-8; **Female** – 9. ARTGC-1, 10. ARTGC-2, 11. ASTGC-10, 12. ASTGC-11, 13. ASTGC-12, 14. ASTGC-14, 15. ASTGC-15, 16. ASTGC-2, 17. ASTGC-3, 18. ASTGC-4, 19. ASTGC-6, 20. ASTGC-8, 21. ASTGC-9, 22. MLTGC-1, 23. MLTGC-10, 24. MLTGC-11, 25. MLTGC-12, 26. MLTGC-13, 27. MLTGC-14, 28. MLTGC-4, 29. MLTGC-7, 30.MLTGC-8, 31. MLTGC-9, 32. MNTGC-1, 33. MNTGC-2, 34. MNTGC-3, 35. MNTGC-4, 36. MNTGC-5, 37. MNTGC-6, 38. MNTGC-8, 39. MZTGC-1, 40. MZTGC-10, 41. MZTGC-11, 42. MZTGC-12, 43. MZTGC-2, 44. MZTGC-3, 45. MZTGC-5, 46. MZTGC-6, 47. MZTGC-9, 48. NLTGC-1, 49. TRTGC-1, 50. TRTGC-10, 51. TRTGC-11, 52. TRTGC-12, 53. TRTGC-13, 54. TRTGC-14, 55. TRTGC-15, 56. TRTGC-16, 57. TRTGC-17, 58. TRTGC-18, 59. TRTGC-19, 60. TRTGC-2, 61. TRTGC-20, 62. TRTGC-21, 63. TRTGC-3, 64. TRTGC-4, 65. TRTGC-5, 66. TRTGC-6, 67. TRTGC-7, 68. TRTGC-8, 69. TRTGC-9, 70. TRTGC-9-1. Geographical origin of the genotypes: AR: Arunachal Pradesh, AS: Assam, ML: Meghalaya, MN: Manipur, MZ: Mizoram, NL: Nagaland, TR: Tripura

Table 6 Additive main effects and multiplicative interaction (AMMI) analysis of variance for fruit traits in teasel gourd across four years

Source	DF	FL			FD			FW		
		Sum Squ	Mean Squ	Contribution (%)	Sum Squ	Mean Squ	Contribution (%)	Sum Squ	Mean Squ	Contribution (%)
ENV	3	0.565	0.188**	0.071	1.843	0.614**	1.568	487.979	162.660**	0.30
REP(ENV)	8	5.660	0.707**	0.715	1.472	0.184**	1.252	832.747	104.093**	0.52
GEN	35	550.144	15.718**	69.496	54.056	1.544**	45.986	118225.008	3377.857**	73.59
GEN: ENV	105	70.551	0.672**	8.912	20.346	0.194**	17.309	14094.165	134.230**	8.77
PC1	37	32.729	0.885**	46.400	11.248	0.304**	55.300	8396.260	226.926**	59.6
PC2	35	26.922	0.769**	38.200	5.324	0.152**	26.200	3489.985	99.714**	24.8
PC3	33	10.901	0.330**	15.500	3.774	0.114**	18.600	2207.920	66.907**	15.7
Residuals	280	94.151	0.336		19.485	0.070		12921.890	46.150	
Total	536	791.621	1.477		117.549	0.219		160655.953	299.731	

*significance at $p \leq 0.05$ **significance at $p \leq 0.01$, DF = degree of freedom, FL = fruit length (cm), FD = fruit diameter, and FW = fruit weight

found to be best with higher germination, i.e., 82.74 and 76.44%, respectively, followed by T_4. However, for the bitter gourd (*Momordica charantia* L.), nutrient media T_4: sucrose (20%), boric acid (25 mg l^{-1}) and calcium nitrate (25 mg l^{-1}) were found to be best with higher (76.20%) pollen germination.

Discussion

Teasel gourd is a high-value and nutritionally superior indigenous cucurbit crop grown widely in the humid subtropical climate of Northeastern India. Due to its wider adoptability, the distribution of the crop ranged from – 11 m (valley) to 887 m (mid-hills) above mean sea level in the region. Due to the higher nutraceutical properties of the fruits, an increase in awareness, and changes in the dietary patterns of consumers, there is an increasing

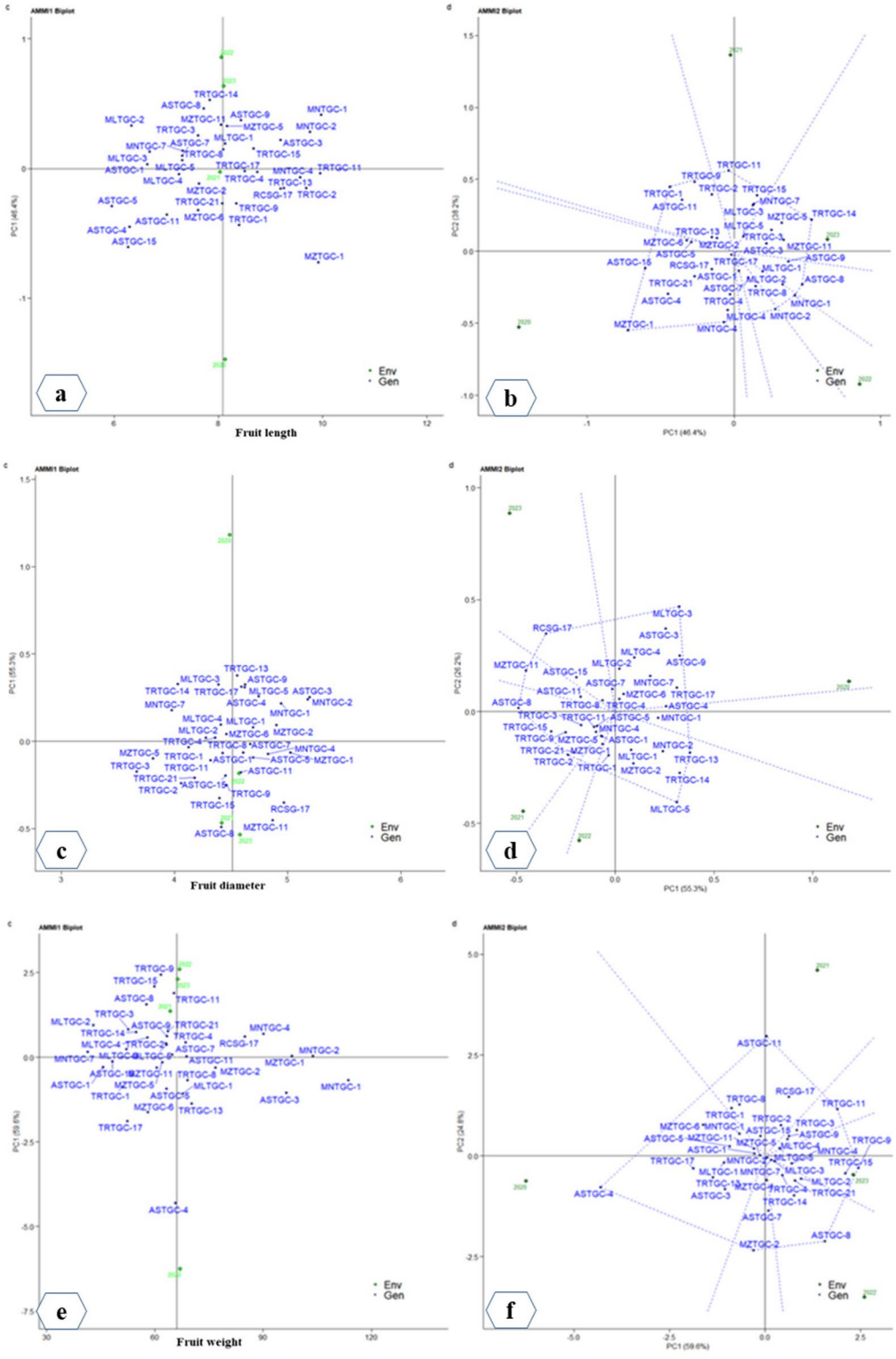

Fig. 8 AMMI Biplot for fruit traits among 36 genotypes of sweet potato over the years/environment using genotypes and environments IPC1 scores (AMMI 1) and IPC2 vs. IPC1 scores (AMMI 2) Where: **a** and **b** for fruit length; **c** and **d** for fruit diameter; **e** and **f** for fruit weight, respectively

Table 7 AMMI stability parameters for fruit traits in teasel gourd across the years

Genotypes	FL			FD			FW		
	Mean	ASI	ASV	Mean	ASI	ASV	Mean	ASI	ASV
ASTGC-1	6.63	0.053	0.141	4.420	0.047	0.179	45.670	0.172	0.692
ASTGC-11	7.01	0.214	0.561	4.590	0.099	0.379	98.730	0.737	2.970
ASTGC-15	6.27	0.285	0.746	4.450	0.116	0.443	48.180	0.143	0.576
ASTGC-3	9.19	0.105	0.275	5.190	0.171	0.653	96.380	0.660	2.660
ASTGC-4	6.29	0.238	0.623	4.740	0.143	0.546	65.700	2.570	10.400
ASTGC-5	5.95	0.137	0.360	4.690	0.054	0.206	63.210	0.553	2.230
ASTGC-7	7.31	0.083	0.218	4.670	0.028	0.106	64.710	0.340	1.370
ASTGC-8	7.72	0.233	0.611	4.410	0.271	1.040	57.600	1.070	4.300
ASTGC-9	8.43	0.174	0.457	4.620	0.191	0.731	63.290	0.390	1.570
MLTGC-1	8.12	0.105	0.274	4.690	0.063	0.241	67.730	0.642	2.590
MLTGC-2	6.33	0.177	0.464	4.280	0.052	0.197	42.920	0.577	2.330
MLTGC-3	6.68	0.136	0.356	4.390	0.217	0.830	52.080	0.144	0.582
MLTGC-4	7.24	0.157	0.411	4.410	0.083	0.316	57.920	0.364	1.470
MLTGC-5	7.30	0.049	0.130	4.620	0.202	0.771	63.170	0.227	0.916
MNTGC-1	9.97	0.224	0.588	4.940	0.121	0.461	113.500	0.423	1.710
MNTGC-2	9.76	0.202	0.529	5.180	0.141	0.540	103.800	0.027	0.110
MNTGC-4	9.57	0.191	0.499	4.820	0.049	0.186	90.080	0.417	1.680
MNTGC-7	7.32	0.141	0.370	3.980	0.107	0.408	41.310	0.098	0.396
MZTGC-1	9.92	0.396	1.040	5.030	0.052	0.200	97.900	0.150	0.605
MZTGC-11	8.05	0.160	0.419	4.870	0.254	0.971	60.530	0.185	0.745
MZTGC-2	7.63	0.064	0.168	4.900	0.079	0.303	76.810	0.608	2.450
MZTGC-5	8.17	0.170	0.444	3.810	0.059	0.227	62.000	0.082	0.332
MZTGC-6	7.61	0.153	0.400	4.460	0.031	0.119	58.060	0.987	3.980
RCSG-17	9.19	0.085	0.223	4.970	0.214	0.819	84.920	0.516	2.080
TRTGC-1	8.39	0.265	0.695	4.120	0.055	0.209	51.050	0.598	2.410
TRTGC-11	9.96	0.215	0.562	4.320	0.062	0.237	65.290	1.160	4.700
TRTGC-13	9.26	0.079	0.208	4.550	0.214	0.818	70.180	0.828	3.340
TRTGC-14	7.83	0.260	0.681	4.030	0.194	0.743	54.820	0.509	2.050
TRTGC-15	8.68	0.166	0.434	4.400	0.181	0.692	59.860	1.250	5.050
TRTGC-17	8.50	0.013	0.033	4.590	0.175	0.667	52.300	1.120	4.530
TRTGC-2	9.69	0.167	0.437	4.060	0.142	0.542	63.030	0.310	1.250
TRTGC-21	8.07	0.141	0.370	4.180	0.125	0.478	63.630	0.485	1.960
TRTGC-3	7.61	0.131	0.343	3.670	0.097	0.371	52.660	0.516	2.080
TRTGC-4	8.74	0.115	0.302	4.360	0.019	0.073	68.540	0.290	1.170
TRTGC-8	8.09	0.115	0.300	4.610	0.038	0.147	69.000	0.511	2.060
TRTGC-9	8.34	0.223	0.585	4.460	0.140	0.537	61.600	1.460	5.870

Where: ASI = AMMI stability Index, ASV = AMMI stability value, FL = fruit length (cm), FD = fruit diameter, and FW = fruit weight

trend in the demand for such high-value crops. Although there is no exact data on the area and production of this crop in the region, in recent years, area expansion has also been observed in the valleys of the rivers Barak and Brahmaputra.

Despite the huge demand, no commercial cultivars are available for this crop. Most farmers grow crops commercially using local landraces maintained and propagated vegetatively through cuttings of sprouted tuberous roots. One cultivar, "Arka Bharath," was recently developed by the ICAR-Indian Institute of Horticultural Research, Bengaluru, through clonal selection. Evaluation of the plant's genetic resources based on agro-morphological traits is the first step in crop improvement. Although there is a broader diversity in the region, there is no research on the extent of genetic diversity based on agro-morphological traits and molecular markers. In the present study, significantly wider variability has been observed for all 24 growth, yield, and quality traits of the fruits. GCV and PCV are the most significant genetic parameters to estimate the extent of variability present in the population. In present study had low (< 10%) GCV and PCV values for SDL and SDW and moderate (10–20%) for LFL, LFW, OL, PL, PDD, FL, FD, HSW, TSS, RS, and VC. As previously noted in the teasel gourd accessions from Assam and West Bengal, India, all other traits, such as FW, SL, SW, PW, STL, STD, PDL, NFPP, YPP, and SDT, showed higher (> 20%) GCV and PCV values

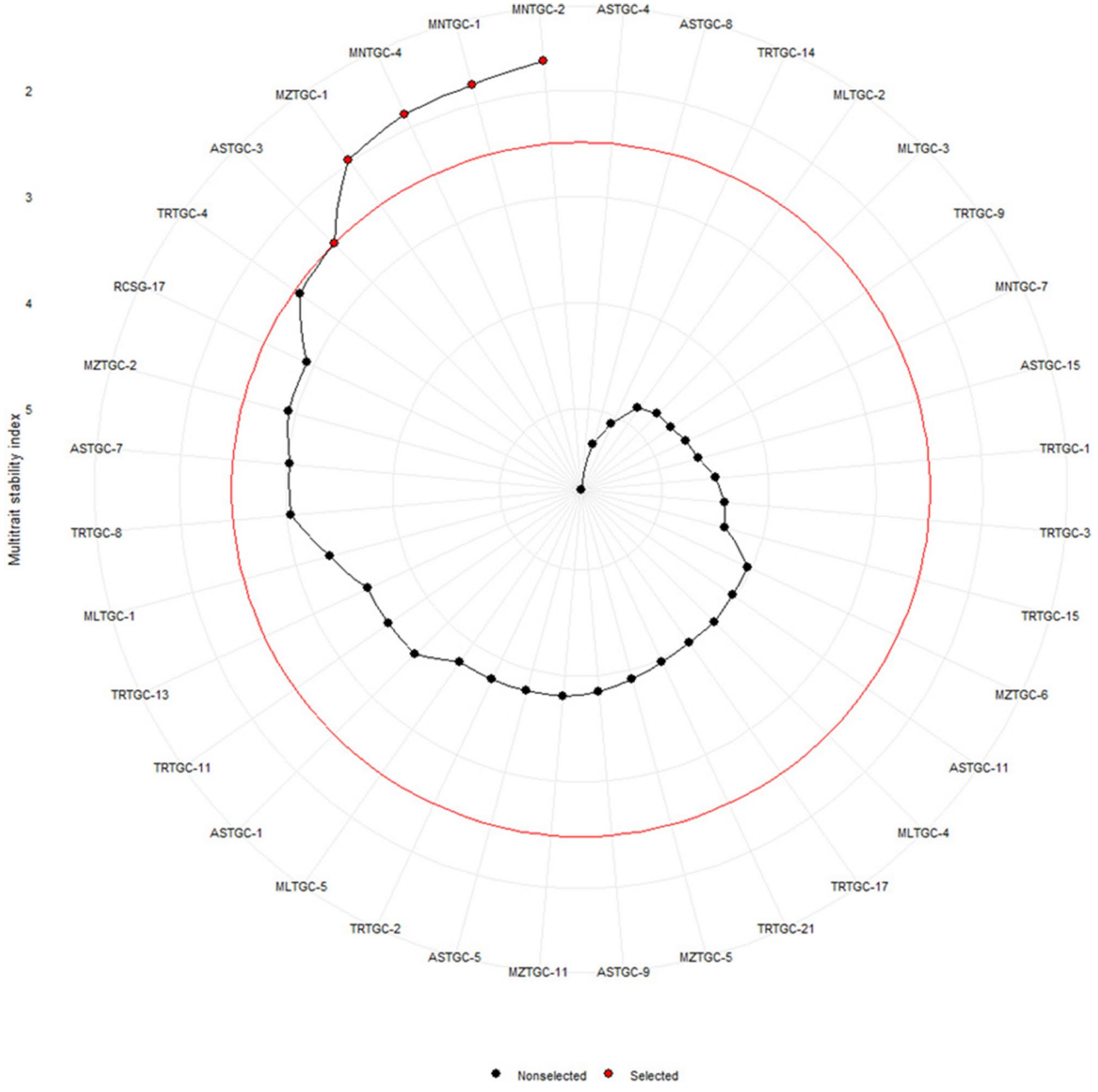

Fig. 9 Ranking of stable genotypes of teasel gourd based on MTSI index at 10% selection intensity

Table 8 Factors linked to correlated traits, selection differential, heritability, and indicators for fruit traits of teasel gourd

Traits	Factor	Indicator	Xo	Xs	SDP	Heritability (%)
FL	FA1	Increase	8.08	9.68	19.8	95.7
FD	FA1	Increase	4.51	5.03	11.5	87.5
FW	FA1	Increase	66.1	100.0	51.9	96.0

Where: Xo = The mean value for the trait in the base population, Xs = The mean value for the trait in a population with selected genotypes, SDP: The selection differential expressed in percentage, FL = fruit length (cm), FD = fruit diameter, and FW = fruit weight

indicated the existence of more variability for these traits [47]. Further, the higher values of PCV over the corresponding values of GCV of all the traits indicate that the observable variation for these traits is not only due to genotypes but also due to the effect of the environment on their expression. The heritability of a trait plays a crucial role in determining genetic advance through selection [48]. The additive gene action is mainly accredited

Table 9 Effect of silver nitrate on flower and fruit traits of the induced monoclinous flowers of teasel gourd

Treatments	FS	FW	FL	FD	DOE
T_0-Control	85.11^a	58.33^a	5.90^a	5.03^a	-
T_1-200 mg l^{-1}	74.33^b	50.17^b	5.17^b	4.67^b	15.67^c
T_2-300 mg l^{-1}	68.22^{bc}	51.33^b	5.17^b	4.70^b	19.67^b
T_3-400 mg l^{-1}	65.33^c	48.33^b	5.20^b	4.53^b	20.00^b
T_4-500 mg l^{-1}	65.00^c	51.67^b	5.30^b	4.63^b	23.67^a
Mean	71.6	51.97	5.35	4.71	21.75
CV (%)	5.04	4.27	2.93	1.98	6.04
CD	6.79	4.17	0.29	0.20	2.97
SE (mean)	2.95	1.81	0.12	0.08	1.28

Where: FS = fruit setting percentage, FW = fruit weight, FL = fruit length (cm), FD = fruit diameter, and DOE = days of efficacy

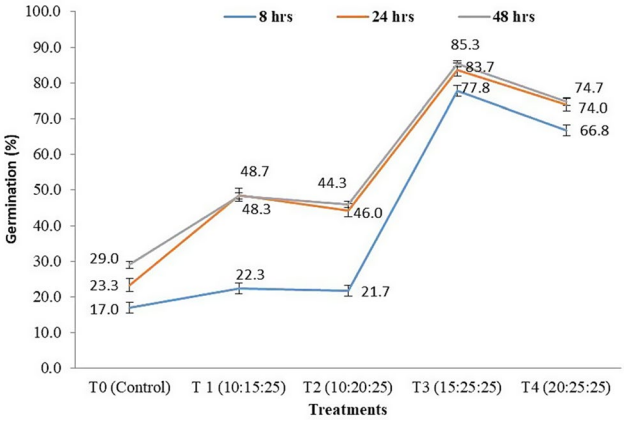

Fig. 11 Effect of nutrients medium on pollen germination in teasel gourd

to high heritability and genetic advancement [49]. Except for SDL, SDW, and FFN, all the other quantitative traits

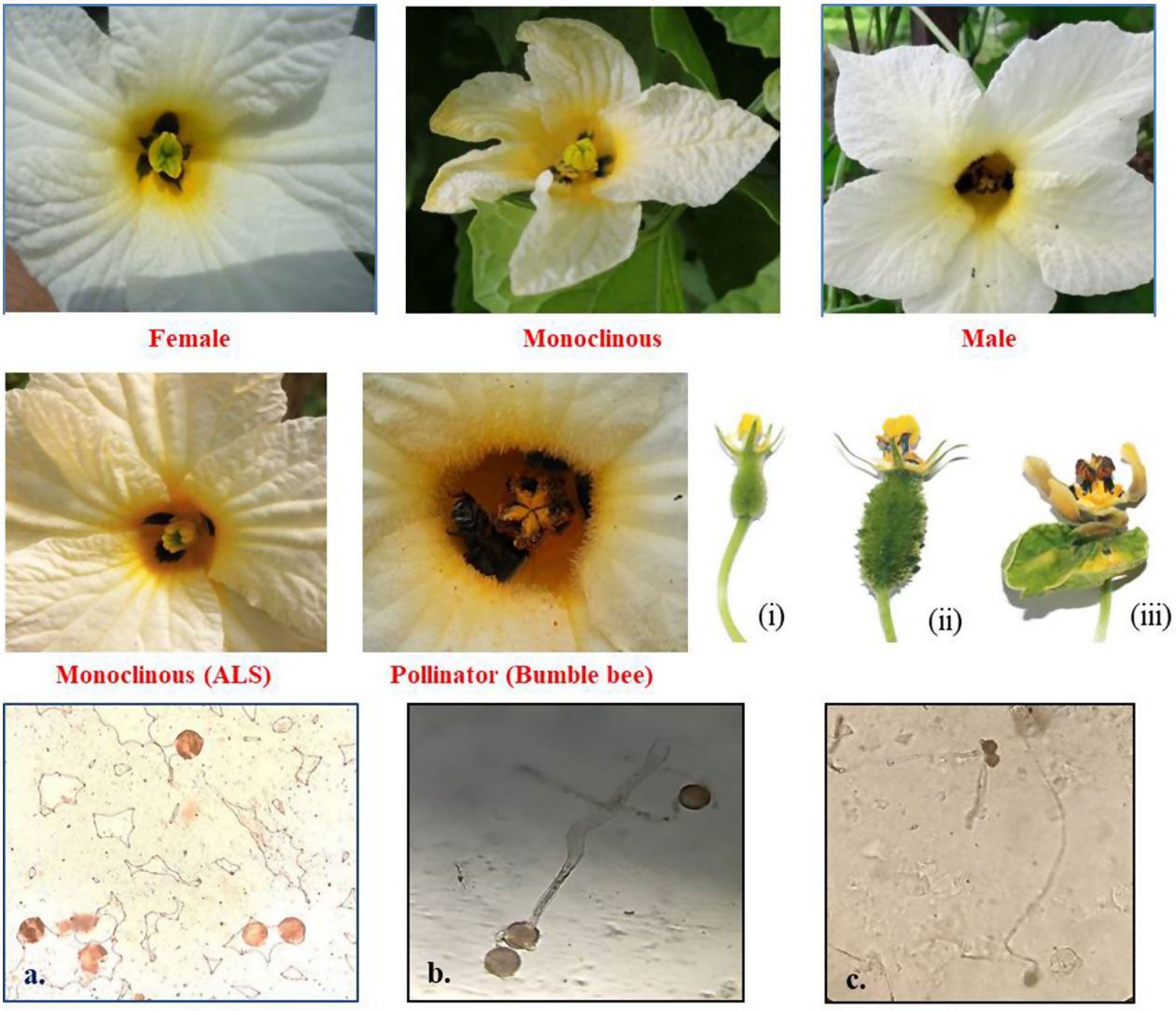

Fig. 10 Male, female, induced hermaphrodite, monoclinous (ALS: Antherless) flowers and flower with pollinator bumble bee of teasel gourd. Flowers without corolla: (i) female, (ii) monoclinous, and (iii) male; Pollen: (**a**) pollen of teasel gourd (male), (**b**) pollen germination from monoclinous, and (**c**) male flowers

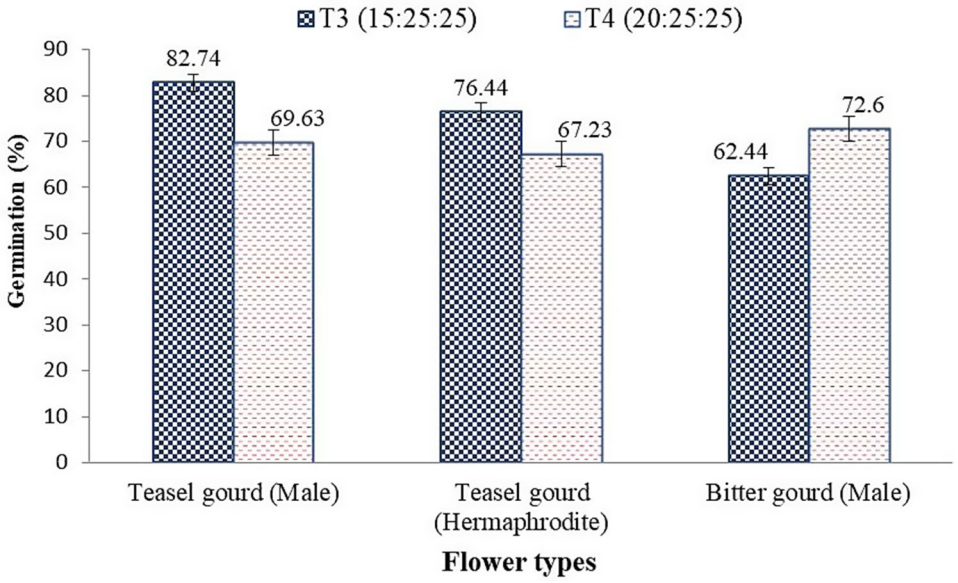

Fig. 12 Effect of selected growing medium on pollen germination in teasel gourd (male and hermaphrodite induced flower) and bitter gourd (male) flowers

of teasel gourd under the study have shown high heritability (> 60%) coupled with high genetic advance (> 20%), indicating that these traits are governed by additive gene action and therefore highly responsive to selection. Similarly, high heritability and moderate to high genetic advances for these traits have also been observed in teasel gourd [6, 50] and closely related species *Momordica dioica* Roxb [51–53].

The accomplishment of any crop improvement program depends on identifying desirable traits and the selection of diverse genotypes [54]. In several crop species, multivariate methods, such as PCA and cluster analysis, have been effectively employed to classify and order the populations based on their genetic variability and phylogenetic relationships. Teasel gourd is a dioecious and cross-pollinated crop, and the diverse genetic resources have evolved in the region's different ecology. Due to vegetative propagation, the heterozygous population is maintained by the growers. Therefore, before starting a breeding program, it is imperative to determine the desired traits, their inheritance, and the diversity of parents. According to Iezzoni and Pritts [55], PCs with an eigenvalue > 1.0 are inherently more informative than any original variable. Also, the results of PCA analysis have indicated the presence of wide variability for different traits. Moreover, the first of eight PCs with an eigenvalue > 1.0 shows the distribution of the variability (71.56%). Similar findings were also observed by Bhagat et al. [56] in the spine gourd. The distribution of the traits over different PCs could be due to poor-relationships, including leaf, fruit, seed, quality, etc. Moreover, the first three PCs accounted for 40.16% of the total variability. PC1 contributed 19.13% of the total variation with loadings > 0.4 and was positively attributed to economically important traits such as FW, YPP, LL, LW, FD, OL, and NFF. The PCA biplot distinguished superior genotypes, such as ASTGC-11, ASTGC-12, MNTGC-3, and TRTGC-9, for the maximum number of traits that could be utilised in commercial production and in further crop improvement programmes.

Further, the cluster analysis results have also shown the presence of substantial diversity among the genotypes of the teasel gourd. The diverse genotypes from clusters with desirable traits can be selected for homosexual hybridisation and selection to get the new recombinant with the maximum desirable characteristics. The genotypes ASTGC-11 and ASTGC-12 from cluster I were superior for fruit size and yield with a maximum cluster mean value, and MNTGC-3 and TRTGC-9 from cluster III were exceptional for yield per plant. The identified superior genotypes for higher yield, such as ASTGC-11 (2.66 kg/plant), MZTGC-3 (1.83 kg/plant), and TRTGC-9-1 (1.80 kg/plant), could be utilized for the commercial production by the farmers under such evaluated conditions as well as further crop improvement by breeders through hybridization with stable genotypes for fruit traits.

Phenotypic along with genotypic data helps in the management of the plant genetic resources, such as the development of the reference sets or core sets and helps to decide the gene bank to supply the germplasm more suited to the breeders or other researchers' needs, quantification of changes during ex-situ/in-situ conservation, identification of redundancy, and gaps in the collections

[57]. SSR markers are most reliable and robust for genotypic analysis due to their presence throughout the genomes, reproducibility, and co-dominance nature. In the present study, the SSR markers selected from other cucurbit species, such as *Momordica charntia* L. and *Sechium edule*, have shown their transferability to teasel gourd (*Momordica subangulata* subspp. *renigera*). The average number of alleles (2.5) per locus indicates the presence of a broad diversity in the teasel gourd population. Moreover, out of 43 markers, only eight have shown heterozygosity in the genotypes of teasel gourd, this could be due to geographical isolation and limited crossing between the populations.

Interestingly, marker Sed-08 from *Sechium edule* was polymorphic and non-heterozygous among these markers. At the same time, it was found to be monomorphic and heterozygous for all the male and female genotypes of the teasel gourd. This could be due to the introgression of the allele in the teasel gourd from some other species unrelated to *Sechium edule*. The observed heterozygosity was comparatively low compared to the expected heterozygosity in the male, female, and overall population. Similarly, Saxena et al. [15] also observed low levels of heterozygosity in *Momordica charantia* L. and Machida-Hirano et al. [43] in *Sechium edule*. This could be due to inbreeding under different geographical origins of the genotypes, limited gene flow with other populations as geographical isolation, selective multiplication from the roots [58–60], and the use of common male parents (10–15%) in commercial production. Hanko et al. [61] also observed low heterozygosity with a higher inbreeding coefficient in the population of the clonally propagated crop *Scutellaria floridana*, a federally threatened Florida-endemic mint. The PIC value range of 0.114 (McSSR-20) to 0.676 (McSSR-6) has also indicated low to higher diversity for the locus. As per the classification of Botstein et al. [62], the average PIC value (0.384) has shown a moderate level of genetic diversity in the population of the teasel gourd. Similar to the PIC value, the moderate level of diversity in the population was also supported by the value (0.468) of average gene diversity and the Shannon information index (0.729, or < 1.5). Moreover, 6 (McSSR-3, McSSR-6, McSSR-86, Sed-01, C-30, and Sed-05) out of 43 polymorphic SSR markers used in the study showed values of the Shannon information index higher than 1.0 (1.083), and their corresponding PIC values above 0.5 (0.580) were found to be most informative for the teasel gourd. Further, markers specific to the male and female genotypes could be utilized for the testing of the hybrid purity in hetero and homosexual hybridization.

According to AMOVA, the least (0.36%) genetic variations were observed between the male and female populations. In comparison, the maximum genetic variation was observed between the genotypes (76.0%), followed by within (24.0%) of the individuals of teasel gourd. Likewise, based on geographical origin, maximum genetic variations have been observed within the population (82.87%), followed by among the population (17.13%) of different geographical origins (states). It has also indicated minimum genetic variation among the male and female populations over the genotypes from different geographical origins. From group-based diversity, the comparatively female population was found to be more diverse than the male population for the number of effective alleles, Shannon's information index, observed as well as expected heterozygosity. This could be due to the selection of diverse female genotypes for their economic importance. The cluster analysis results have also revealed substantial diversity in male and female genotypes of the teasel gourd. The results of the PCoA and genetic structure analyses also support this. The rate of genotypes with admixture was 25% in males and 37% in females (at the 95% significance level). As per geographical origin, the proportion of admixture was higher in genotypes from Meghalaya, Mizoram and Tripura. The maximum admixture in the genotypes of Meghalaya may be due to the sexual origin from the seeds. This could be due to the natural crossing or free gene flow between populations nearby by the pollinators. The genotypes from Assam (Barak Valley), Mizoram, and Manipur were found close. The genotypes of teasel gourd have probably migrated from the valley to the hills of Manipur, Meghalaya, and Mizoram.

Humid-subtropical climates are considered ideal for teasel gourd production; the soil and environment of growing locations have a significant impact on fruit yield and quality. Finding a stable genotype is particularly crucial for commercial production. In the context of the region's humid subtropical climates, our research examined the AMMI models for the stability of fruit traits. Analysis of variance (ANOVA) has demonstrated the noteworthy impact of both genotypes and the genotype × environment interaction on the expression of all the fruit attributes over the years in the mega-environment. From stability parameters analysis, none of the genotypes were found stable for all the traits; similar findings were also observed in other vegetatively propagated crops, such as sweet potato [63] and guava [54]. This could be due to the effect of varied weather parameters (temperature and rainfall) over the years (Supplementary Fig. 2). In northeastern India, teasel gourd is mostly grown as a rainfed crop during the rainy season. Among the weather parameters, the rainfall has been observed as an important factor with a higher average coefficient of variation (63.25%) over the years (2019–2022), and it ranges from 26.94% (June) to 132.32% (February). Comparatively, temperature has shown lower coefficients of variation of 5.71 and

12.12% for average minimum and maximum temperatures, respectively.

AMMI biplot differentiated the stable genotypes for different traits, and based on ASV and ASI values, genotypes MNTGC-2, MZTGC-5, and MNTGC-7 were found to be most stable for average fruit weight over the years. MTSI is widely used to identify stable genotypes for multiple traits in different crop species. Based on MTSI analysis, genotypes MNTGC-2, MNTGC-1, MNTGC-4, MZTGC-1, and ASTGC-3 were found to be most stable considering all the fruit traits (FL, FD, and FW) at 10% selection intensity. These genotypes can be utilised for commercial production and for further crop improvement. The high heritability derived by the factor analysis has supported improvement through selection.

Dioecism is one of the most limiting factors in exploiting heterosis in the teasel gourd. Besides, the genetic diversity of the male parents and their contribution to the progeny remain unknown. Therefore, hybridisation between homosexual hybrids (induced monoclinous flowers using $AgNO_3$) has been attempted by some researchers in sweet gourd [11]. The crop is commercially propagated through cuttings of sprouted roots, which provide scope for the fixation and utilisation of the heterosis in crops like teasel gourd. In the present study, to standardise the concentration of $AgNO_3$ for induction of hermaphroditism, the fruit setting (FS) percentage decreased with an increase in the concentration of $AgNO_3$, and FS was at its maximum (85.11%) under control conditions. The number of deformed flowers (poorly developed ovary) was also observed with an increase in concentration for initial flowers during 7–10 days after application, and this abnormality could be due to inhibition in the synthesis of the hormones responsible for the development of the ovary in the pre-spray flower bud differentiation. The optimum concentration of $AgNO_3$ was recorded at 500 mg l^{-1} for a maximum day of efficacy (23.67 days). Through induced hermaphroditism and selfing, the seed production companies can multiply the high-yielding female genotypes sexually for commercial seed production, as there will be no segregation for the male and female in the progenies. We also observed variations in the efficacy period for plant age and weather parameters like temperature and rainfall over the years, indicating the need for further research.

The pollen germination (PG) in the teasel gourd under the control conditions is very low (17–29%); however, it can be increased to 85% using a nutrient solution. The nutrients, such as calcium (Ca) and boron (B), are essential for pollen grain development, germination, and pollen tube growth [64]. Calcium is involved in cationic balance and is necessary for tube elongation [65]. Calcium uptake across the plasma membrane, exocytotic activity, and the biosynthesis of cell wall components are all processes that occur in the tip region of pollen tubes and control pollen tube development [66–67]. Boron combines with sugar to form sugar-borate complexes, which promote absorption, translocation, and the metabolism of sugars in the pollen [64]. In the present study, the compositions of the nutrients sucrose, calcium, and B have shown significant effects on PG of the teasel gourd. Among the treatments, the best nutrient medium was identified as a combination of sucrose (15%), boric acid (25 mg l^{-1}), and calcium nitrate (25 mg l^{-1}), which gave the highest PG at 12, 24, and 48 h. after culturing. This treatment combination (T_3) has increased (82.3%) the average PG across the incubation period by 256.13%, followed by treatment T_4, i.e., sucrose (20%) + boric acid (25 mg l^{-1}) + calcium nitrate (25 mg l^{-1}). This could be due to the positive effect of nutrients sucrose, boric acid, and calcium nitrate on pollen growth and development. Similarly, the higher germination (66%) was also observed by Rathod et al. [28] in *Momordica* species from the growing nutrient media comprised of sucrose at 15% + boric acid at 0.25% and calcium nitrite at 300 mg l^{-1}. The pollen from males, as well as induced monoclinous flowers of teasel gourd (*Momordica subangulata* subspp. *renigera*) and bitter gourd (*Momordica charantia* L.), have shown a differential response to the growing nutrients composition to the pollen. For male and induced monoclinous pollen of teasel gourd treatments T_3: sucrose (15%), boric acid (25 mg l^{-1}), and calcium nitrate (25 mg l^{-1}) were found to be best with higher germination, i.e., 82.74 and 76.44%, respectively, followed by T_4. However, for the bitter gourd, nutrient media T_4 with a higher sucrose concentration (20%) was best with higher PG (76.20%). The above results indicate enormous scope to enhance the fruit setting in teasel gourd by improving PG. These identified nutrient media can be used to efficiently utilise the pollen to improve the fruit setting under the hybridisation programme.

Conclusion

The findings of the above study have indicated that, due to its wider adaptability, there is a vast potential for area expansion under such a high-value crop, teasel gourd, in the northeastern states of India. The region has wider variability for growth, yield, and quality attributes. The results of molecular analysis have also indicated the presence of substantial genetic diversity in the male and female populations of the crop. The economic traits, such as fruit yield and quality attributes, showed high heritability and genetic advancement under the influence of additive gene action and suggested improvement in these traits through selection. The farmers of the region can utilize the high-yielding superior genotypes such as ASTGC-11, MZTGC-3, and TRTGC-9-1 for commercial production using the sprouted root cuttings.The

fruit traits such as FL, FD, and FW were also significantly affected by the genotype and genotype × environmental interaction. The MTSI identified the superior and stable genotypes MNTGC-2, MNTGC-1, MNTGC-4, MZTGC-1, and ASTGC-3 that could be used for further crop improvement. Heterosis can also be exploited by hybridization between induced monoclinous /hermaphrodite females by foliar sprays of silver nitrate at 500 mg l^{-1}. Since silver nitrate is toxic in nature, the seed production companies can use it for commercial seed production of the superior female genotypes through induction of hermaphroditism and selfing under the proper supervision of the experts. Further, the mechanism of genetic regulation in sex determination can also be explored by transcriptome analysis of the control and silver nitrate-treated plants, which will open the window for genetic modification using modern genome editing tools like CRISPR-Cas9 to produce plants of the hermaphrodite type. Moreover, the PG can be enhanced significantly by the use of nutrient media comprised of sucrose (15%), boric acid (25 mg l^{-1}), and calcium nitrate (25 mg l^{-1}).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06400-5.

Supplementary Material 1: Table 1. Passport data of the teasel gourd germplasm collections. Table 2. Mean performance of 62 female genotypes of teasel gourd for 24 different growth, yield and quality traits. Fig. 1. Gel profile of the SSR marker McSSR-16 showing allelic variations among the 70 genotypes of the teasel gourd. Fig. 2. Weather parameters of the evaluation site (Umiam) over the years (2019-2022). a) Average minimum and maximum temperature, b) Coefficient of variations for temperature, c) monthly rainfall over the years, d) Coefficient of variations for rainfall.

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Author contributions
Conceptualization, V.K.V.; methodology, V.K.V., H.R., and A.K.; validation, V.K.V., A.K., and H.R.; data curation, V.K.V., A.K., A.P. and M.B.D.; writing—preparation of the original draft, V.K.V.; writing—review and editing, H.R., A.P., P.B., S.H. and V.K.M.; acquisition of funds, V.K.V. and A.P. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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