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RESEARCH PAPER

Amelioration of economically important traits through mutagenesis in spine gourd (*Momordica dioica*)

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Abstract

Mutation breeding can sometimes be endeavored along with conventional breeding efforts, resulting in the discovery of some economically useful genetic changes. Evaluation of 96 first-generation mutant germplasm lines of spine gourd (*Momordica dioica* Roxb.) was done through morphological descriptors and molecular makers. Correlation coefficient analysis, path analysis and cluster analysis supplemented by molecular marker-based population structure analysis helped in understanding the population behavior and identification of high-yielding lines. The yield was found to be highly positively correlated with days to first flowering, leaf width, single fruit weight and number of fruits per plant. Also, the maximum positive direct effect on yield was shown by single fruit weight followed by the number of fruits per plant and leaf width. The population structure analysis revealed 4 clusters of population. Few high-yielding lines were identified which approximately yielded 1.5 times more than the checks. Further refinement in these mutant lines through breeding programs coupled with in-depth molecular analysis can lay the foundation of the release of some excellent spine gourd varieties in the future.

Keywords: Mutation breeding, Spine gourd, Genetic evaluation, Variability assessment.

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Introduction

Spine gourd (*Momordica dioica* Roxb.) is a flowering plant in the Cucurbitaceae family. It is a rhizomatous, perennial, dioecious vegetable crop. Along with being highly nutritious, it also has pharmaceutically important compounds. It is distributed in India, Bangladesh, China, Malaysia, Nepal, Myanmar, Pakistan and Sri Lanka, etc (Tiwari *et al*., 2022). In India, it is widely adopted in versatile soil and climatic conditions of different states (Rakh & Chaudhari, 2010). Evaluation of mutant lines in spine gourd was attempted with the aim of discovering some revolutionary yield attributing traits or alleles that can upscale the position of spine gourd in mainstream commercial cultivation. An attempt in mutation breeding was made by Tiwari *et al*. (2019) by treating the seeds of a released variety Indira Kankoda-1 with acute gamma radiation at the doses of 0, 50, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325 and 350 Gy at the Bhabha Atomic Research Center (BARC), Mumbai, India and performed germination test of the treated seeds at the Research cum instructional farm, RMD college of Agriculture and Research Station, Ambikapur. The objective of the current study was to evaluate the mutant population using phenotypic characters and genotypic markers.

Materials and Methods

Experimental site

The investigation was conducted at the research cum institutional farm and Plant Molecular Biology and Biotechnology Laboratory at Raj Mohini Devi College of Agriculture and Research Station, Ambikapur, Chhattisgarh during *kharif* 2022-2023. The place of research is a sub-humid region.

Experimental materials

The present study was performed on M_1 (first mutant generation) population. The population was obtained from the existing tubers available at the research farm under the AIRCN on potential crops at Ambikapur center. The experimental material consisted of 96 mutant lines along with three released varieties Indira Kakoda 1, Indira Kakoda 2 and Chhattisgarh Kakoda 2 as checks. The experiment was conducted in augmented block design. The planting geometry was 2m x 2m and the ratio of 8:1 (Female: Male) was monitored and maintained for good fruit yield. Observations of various characters based on the parameters were recorded throughout the growing season. The parameters for morphological observation are given in Table 1. The recorded data was then analyzed for character association. The character association was carried out through correlation and path analysis. A cluster diagram of the lines using morphological data was also constructed. For assessing the genetic diversity of the mutant lines, molecular genotyping was performed using microsatellite (SSR) markers, which included DNA isolation, quantification, dilution of DNA, PCR amplification using SSR primers, electrophoresis using agarose gel, and documentation of gel by using E-BOX CX5.TS (20M). The generated molecular data was then used for scoring of the markers and estimation of the Polymorphic Information Content (PIC) of the primers. The list of primers used for molecular characterization is given in Table 2.

The data generated through the molecular analysis was subjected to population structure analysis to determine the nature and clustering of the population. The analysis detected the actual number of categorizations of the population (denoted as K). The run length was specified as being made up of 100000 burning periods and 100000 replications. Plotting the mean estimate of the log posterior probability of the data L (K) against the specified K value revealed the ideal k value. The maximum value of L (K) was used to determine the true number of sub-populations.

Statistical procedures

For the estimation of association between the various yield attributing traits correlation and path analysis were carried out. The correlation coefficient (r) was calculated for all possible combinations of fruit yield and its component

Table 1: Parameters for morphological characterization in spine gourd

S. No.	*Characteristics*	*States*	*Parameter*	*Stage of observation*
1	Days of first flowering	Early (<30 days)	1	Vegetative
		Medium early (30-40 days)	3	
		Medium (40-60 days)	5	
		Late (>60 days)	7	
2	Number of first flowering node	1-10 nodes	1	Vegetative
		11-20 nodes	2	
		21-30 nodes	3	
		31-40 nodes	4	
		41-50 nodes	5	
		51-60 nodes	6	
		61-70 nodes	7	
3	Stem colour	Light green (L.G)	1	Vegetative
		Green (G)	3	
		Dark green (D.G)	5	
4	No. of ridge on stem	Present (5 ridge)	1	Vegetative
		Absence no ridge	9	
5	Node colour at the attachment of the leaves	Light green (L.G)	1	Vegetative
		Green (G)	3	
		Dark green (D.G.)	5	
		Blackish green (B.G)	7	
6	No. stem per plant	Few (<5 stems)	1	Vegetative
		Moderate (5-10 stems)	5	
		Many (>10 stems)	9	
7	Leaf colour	Light green (L.G)	1	Vegetative
		Green (G.)	3	
		Dark green (D.G.)	5	
8	Leaf length	Short (1-7 cm)	1	Flowering
		Medium (7.1-15 cm)	5	
		Long (>15.1 cm)	9	
9	Leaf width	Narrow (1-7 cm)	1	Flowering
		Medium (7.1-15 cm)	5	
		Wide (>15.1 cm)	9	
10	Leaf margin	Absent (no attachment)	1	Flowering
		Slightly dented (SD)	3	
		Medium dented (MD)	5	
		Dented (D)	7	
		Serrated (5 lobes)	9	
11	Leaf pubescence	Absence (Ab)	1	Flowering
		Few (F)	3	
		Medium (M)	5	
		More (Mo)	7	
12	Pedicel length	Short (1-5 cm)	1	Flowering
		Medium (5.1-15 cm)	5	
		Long (>15.1 cm)	9	
13	Ovary length	Short (<10 mm)	1	Flowering
		Medium (11-20 mm)	5	
		Long (>21 mm)	9	

14	Ovary diameter	Small (<7mm)	1	Flowering
		Large(>7.1mm)	5	
15	Style length	Short (<6mm)	1	Flowering
		Medium (6.1-9 mm)	5	
		Long (>9.1 mm)	9	
16	Pistil tip length	Short (<4mm)	1	Flowering
		Medium (4.1-6.0mm)	5	
		Long (>6.1 mm)	9	
17	Fruit colour	Greenish yellow (GY)	1	Fruiting
		Yellow green (YG)	3	
		Green (G)	5	
		Dark green (DG)	7	
		Light green orange (LG)	9	
		Light orange (LO)	11	
18	Fruit shape	Round (R)	1	Fruiting
		Oval (OV)	3	
		Cylindrical (C)	5	
		Oblong (O)	7	
		Spindle (S)	9	
19	Conical spine density	Thin (T)	1	Fruiting
		Thick (TK)	3	
20	Conical spine strength	Soft (S)	1	Fruiting
		Hard (H)	3	
21	Pedicel attachment with the fruit	Depressed (D)	1	Fruiting
		Slightly depressed (SD)	3	
		Pointed (P)	5	
22	Fruit length	Short (<5 cm)	1	Fruiting
		Medium (5.1-9 cm)	3	
		Long (9.1-13 cm)	5	
		Very long(>13.1 cm)	7	
23	Fruit diameter	Short (<5 cm)	1	Fruiting
		Medium (5.1-9 cm)	3	
		Long (9.1-13 cm)	5	
		Very long(>13.1 cm)	7	
24	Single fruit weight	Light (1-5 g)	1	Fruiting
		Medium light (5.1 – 10 g)	3	
		Medium (10.1 – 15 g)	5	
		Heavy (15.1 - 20 g)	7	
		Very heavy (>20 g)	9	
25	No. of fruits per plant	Very Few (<20)	1	Fruiting
		Few (21-41)	3	
		Moderate (41-60)	5	
		Many (61-80)	7	
		Profuse (81-100)	9	
		Very Profuse (>100)	11	
26	Fruit yield per plant	Low (<1.0 kg)	1	Harvesting
		Medium (1.1-2.0 kg)	3	
		Good (2.1-3.0 kg)	5	
		Very good (3.1-4.0 kg)	7	
		Bumper (>4.1 kg)	9	
27	Yield (qt/hac)	Low (<10)	1	Harvesting
		Less (11-20)	3	
		Medium (21-30)	5	
		High (30-40)	7	
		Very high (41-50)	9	
		Bumper (>50)	11	
28	No seed per fruit	Few (1-10)	1	Harvesting
		Less (11-20)	3	
		Medium (21-30)	5	
		Many (>30)	7	
29	100 Seed weight	Light (<10 g)	1	Harvesting
		Medium bold (10-30 g)	3	
		Bold (>30 g)	5	

Table 2: List of primers used for molecular analysis

S. No.	*Primer*	*Sequence*		*Amplified Temp. (°C)*
1.	SgSSR3	F	CAGCACTCAGTCTTAAAACAA	54.2
		R	TGAATAATATTGCACCCACTC	
2.	SgSSR4	F	CAGCACTCAGTCTTAAAACAA	54.1
		R	TGAATAATATTGCACCCACTC	
3.	SgSSR12	F	TGAGTAAGAGAGAGAACGAAAA	55.3
		R	TGCAGCATATACGAACAAGTA	
4.	SgSSR18	F	ATAGAGAAATTGGGTGGAAGA	54.0
		R	AAAACCCAAGTCTCAATTCTC	
5.	McN1	F	GTCTTCCAGGTTGGGAACAG	57.1
		R	ATCTGGTTCCTCGGGAGATT	
6.	McN5	F	CGTCGCTCTCACAAGAGATAAG	57.0
		R	TTTGGTGGAAATCCCCTATT	
7.	SgSSR22	F	CATAAGTGCATGTGCGTATAA	54.1
		R	TGTGCATGTCTGTGTTTGTAT	
8.	SgSSR23	F	CACTTTTTGATTCCCTTTCTC	54.0
		R	GAGCATATAGACCCGAACAC	
9.	SgSSR24	F	CACTTTTTGATTCCCTTTCTC	54.0
		R	AAAGTGAGGAAGAATATGTGG	
10.	SgSSR25	F	GACTCAATGGAAGCTGTTCTA	56.0
		R	GGGTCGTCATTAATCAAATAG	
11.	SgSSR9	F	TTTTAGTTTCCTCATGGATTG	54.0
		R	CACACAGAGAGACCATCAAAT	
12.	SgSSR10	F	CTCATTTCTTGGAAGCTACG	54.2
		R	CATGTGATGGAATTGAACTTT	

13.	SgSSR12	F	TGAGTAAGAGAGAGAACGAAAA	54.9
		R	TGCAGCATATACGAACAAGTA	
14.	SgSSR14	F	CTACTGGAAACAACATGGAAG	52.0
		R	AAGCTGACTCCAAGAAATGAT	
15.	SgSSR16	F	AGGTTTGAAAATAAGTGCCTA	54.0
		R	TGAAGAGAAGATGAAGATGGA	
16.	SgSSR17	F	AGAGATAACCGCAGTTCATAA	54.1
		R	TTCTCTCTTTTCTCTCATCCA	
17.	SgSSR11	F	CACATGATTTATGGGTTTCAT	52.0
		R	GCATTAATTTTGGGTAAGGAT	
18.	SgSSR13	F	ATTGGTCATCTCGAAAGGTAT	54.1
		R	GTTGGAAAAATGTGGTAACAG	
19.	McN12	F	CAGAGGGGTGGTTCCTCTTT	57.0
		R	CCACATGGATGATCGAGAGA	
20	McN24	F	CTCCAACTTGAGGAAAGAAAAC	57.1

parameters by using the standard procedure given by Searle (1961) and the path coefficient was calculated separately for all important characters considering fruit yield as a dependable variable. Path-coefficient was estimated using simultaneous equations and the equations showed a basic relationship between correlation coefficient and path-coefficient.

Results and Discussion

The mean of observations of all the characters for each genotype is given in the supplementary file 1. The lines showed high variability for the characters. The variability among the mutant lines with respect to quantitative characters is presented in the form of a mean table in Table 3. The lines showed high variability for the characters. The cluster diagram generated using the morphological data (Figure 1) revealed that initially the genotypes were divided into 2 clusters at a similarity 0.73. Cluster I consisted of 29 genotypes and cluster II consisted of 70 genotypes. Cluster I was further divided into 2 sub-clusters (cluster IA and IB) having 14 and 15 genotypes respectively at the similarity of 0.80 and cluster II was further divided into 2 sub-clusters (clusters IIA and IIB) having 4 and 66 genotypes respectively at the similarity 0.79. The total similarity ranged from 0.73 to 0.96. Among all the genotypes, the highest similarity was observed between CP 107 and CP 157. The most divergent genotypes were M2 4 and CP 182 with a similarity 0.73. A cluster analysis was also generated based on molecular analysis, given in Figure 2. In the molecular data-based cluster analysis, the population was divided into 2 clusters at a similarity 0.510 with a cluster range of 0.510 to 1. The most distant genotypes were CP 143 and M2 151, and 18 genotype groups were found to have similarity 1.

The best-performing mutant lines with respect to the check varieties for each character has been listed in Table 4.

Table 3: Analysis of quantitative morphological data

Characters	*Mean*	*Min*	*Max*	*Std. dev.*	*CV (%)*
Days of 1st flowering	37.53	31.00	47.00	4.52	12.04
Number of 1st flowering nodes	14.14	9.00	22.00	3.48	24.61
Number of stem per plant	5.58	3	8	1.46	26.13
Leaf length (cm)	8.06	3.18	21.98	3.10	38.44
Leaf width (cm)	6.04	2.43	11.44	2.09	34.66
Pedicel length (cm)	3.37	1.74	9.21	1.36	40.43
Ovary length (mm)	23.06	16.40	31.60	2.82	12.22
Ovary diameter (mm)	5.92	3.80	8.00	0.94	15.94
Style length (mm)	5.48	3.60	8.00	0.95	17.28
Pistil tip length (mm)	3.49	2.20	6.40	1.01	28.97
Fruit length (cm)	5.67	3.62	7.92	0.86	15.25
Fruit diameter (cm)	3.77	2.82	5.38	0.55	14.67
Single fruit wt. (g)	15.06	5.83	33.56	5.82	38.62
Number of fruits/ plants	78.85	27.00	166.00	29.20	37.04
Fruit yield/plant (kg)	1.26	0.26	3.46	0.75	60.00
Yield (quintal/hac)	27.94	5.68	76.81	16.76	60.00
Number of seeds/ fruit	23.64	1.00	38.00	6.73	28.48
100 seed wt. (g)	9.42	1.20	17.57	2.01	21.32

Average Single fruit weight (g) of the checks IK 1 (20.44), IK 2 (19.78) and CK (18.85) were quite lower than the lines CP 57 (27.86), CP 61 (25.22), CP 130 (33.56), CP 134 (27.89), CP 145 (25.23) and CP 154 (28.23). The number of fruits per plant of the lines CP 43 (121.00), CP 48 (109.00), CP 84 (110.00), CP 128 (166.00), CP 157 (112.00) and CP 182 (116.00) were comparable (or slightly higher) than the checks IK 1 (100), IK 2 (113) and CK 2 (91). Yield (quintal/ha) of the lines CP 57 (58.81), CP 84 (59.03), CP 128 (64.77), CP 130 (76.81), CP 134 (66.31) and CP 154 (60.85) were quite better than the checks IK 1 (45.41), IK 2 (49.67) and CK (38.11).

Correlation Analysis

Days of 1st flowering was positively correlated with single fruit weight (0.598), number of fruits per plant (0.732) and yield (0.779). The number of 1st flowering nodes was positively correlated with single fruit weight (0.392), number of fruits per plant (0.371), and yield (0.428) Leaf length was positively correlated with single fruit weight (0.561), number of fruits per plant (0.560) and yield (0.635). Ovary length was positively correlated with single fruit weight (0.514) , number

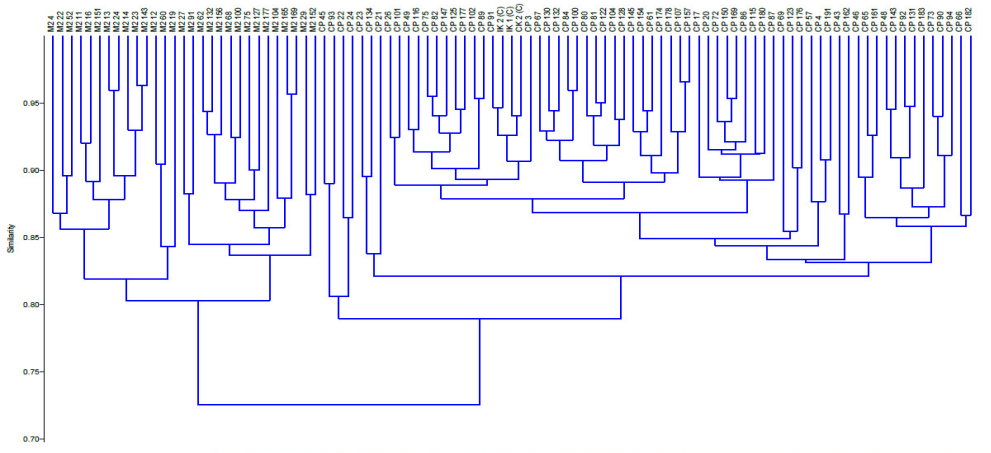

Figure 1: Dendrogram of genotypes based on morphological data

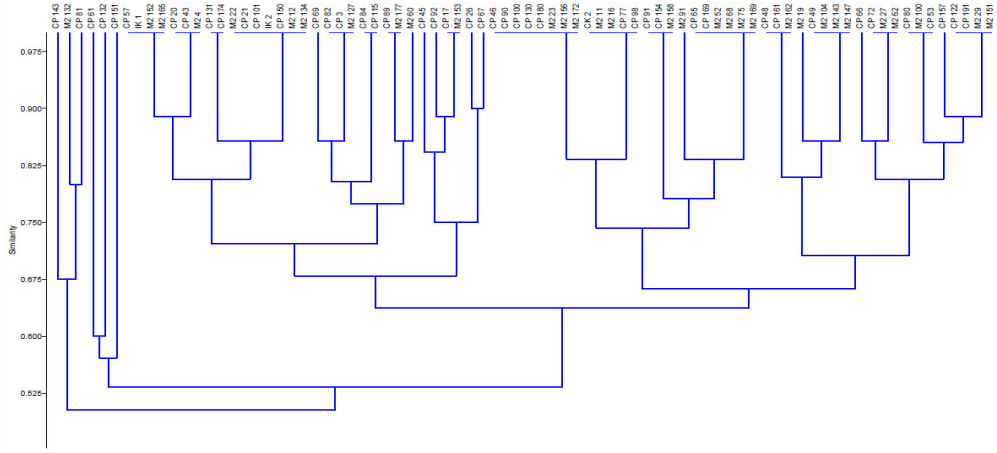

Figure 2: Dendrogram of genotypes based on molecular data

of fruits per plant (0.644), and yield (0.636). Fruit length was positively correlated with single fruit weight (0.374), and yield (0.301). Single fruit weight was positively correlated with the number of fruit plant (0.437), and yield (0.891). The number of fruits per plant was positively correlated with yield (0.768) and the number of seed per fruit was found positively correlated with yield (0.638). The correlation coefficient values for the characters are given in Table 5. Yield was found to be highly positively correlated with days to first flowering, leaf width, single fruit weight and number of fruits per plant. Thus, selection for these four characters can be useful in yield improvement. A similar high correlation between the number of fruits per plant and yield were found by Srivastava and Srivastava (1976), while performing genetic analysis for 10 bitter gourd (*Momordica charantia*) lines. Also in a study conducted by Saranyadevi *et al*. (2017) on mithipagal genotypes (*Momordica charantia var. muricata*) similar correlations were observed. Their correlation analysis revealed that fruit yield per vine was significant and positively correlated with nodes of female flower appearance, fruits per vine, fruit length, fruit weight and seeds per fruit.

Path Analysis

The path analysis revealed that days of 1st flowering (0.093), leaf width (0.102), pistil tip length (0.068), fruit length (0.007), average fruit diameter (0.017), single fruit weight (0.660), number of fruit/plant (0.492), and 100 seed weight (0.008) had a positive direct effect on the yield. The highest positive effect was exerted by single fruit weight (0.660) followed by number of fruit/plant (0.492) and lowest by fruit length (0.007). The characters like number of 1st flowering nodes (-0.046), leaf length (-0.086), pedicel length (-0.038), ovary length (-0.013), ovary diameter (-0.028), style length (-0.013) and number of seed/fruit (-0.023) showed negative direct effects on the yield. The highest negative effect was shown by leaf length (-0.086) and lowest by both ovary length (-0.013) and style length (-0.013). The effect of residual factor (0.0212) on fruit yield per ha was negligible, thereby, suggesting that no other major yield component is left over. The direct and indirect effects of the characters on yield are given in Table 6. The maximum positive direct effect was shown by single fruit weight followed by a number of fruits per plant. Similar results were found by Pathak *et al*. (2014),

Table 4: Identification of better genotypes with reference to checks

Character	*Check performance*	*Best/better performing lines*
Days of 1st flowering	IK 1 (38), IK 2 (38), CK 2 (39)	CP 84 (46), CP 128 (47), CP 130 (47)
Number of 1st flowering nodes	IK 1 (17), IK 2 (14), CK 2 (15)	CP 100 (18), CP 49 (19), CP 69 (19), CP 131 (20), CP 132 (20), CP 132 (20)
Leaf length (cm)	IK 1 (6.65), IK 2 (6.95), CK 2 (7.04)	CP 21 (21.98), CP 122 (13.66), CP 43 (15.58), CP 128 (13.56), CP 130 (14.36), CP 100 (13.26)
Leaf width (cm)	IK 1 (4.51), IK 2 (4.77), CK 2 (4.30)	CP 100 (10.32), CP 104 (10.32), CP 122 (11.16), CP 128 (11.44), CP 130 (10.32), CP 132 (10.94)
Pedicel length (cm)	IK 1 (8.21), IK 2(9.21), CK 2 (8.90)	No genotypes were found to have better pedicel length than the checks.
Ovary length (mm)	IK 1 (22.31), IK 2 (22.11), CK 2 (22.85)	CP 86 (29.00), CP 100 (31.20), CP 101 (31.60), CP 147 (26.80), CP 87 (27.20), CP 69 (27.40)
Ovary diameter (mm)	IK 1 (5.74), IK 2 (5.86), CK2 (5.90)	CP 4 (7.60), CP 45 (8.00), M2 132 (7.60), M2 165 (7.80), M2 100 (7.40), M2 104 (7.40)
Style length (mm)	IK 1 (5.19), IK 2 (5.39), CK2 (5.69)	M2 12 (7.60), M2 60 (7.80), M2 62 (7.60), M2 104 (7.60), M2 152 (8.00) M2 156 (7.60)
Pistil tip length (mm)	IK 1 (3.27), IK 2 (3.50), CK 2 (3.19)	M2 156 (5.90), M2 165 (6.00), M2 169 (6.00), M2 152 (6.40), M2 104 (6.10), M2 60 (6.00)
Fruit length (cm)	IK 1 (5.60), IK 2 (5.49), CK 2 (5.23)	CP 84 (7.12), CP 86 (7.26), CP 104 (7.86), CP 125 (7.24), CP 130 (7.92), CP 132 (6.98)
Fruit diameter (cm)	IK 1 (4.09), IK 2 (4.31), CK 2 (4.30)	CP 61 (4.88), CP 91 (5.38), CP 123 (4.66), CP 130 (5.38), CP 162 (5.32), CP 191 (4.52)
Single fruit wt. (g)	IK 1 (20.44), IK 2 (19.78) and CK (18.85)	CP 57 (27.86), CP 61 (25.22), CP 130 (33.56), CP 134 (27.89), CP 145 (25.23), CP 154 (28.23)
Number of fruits/plant	IK 1 (100), IK 2 (113), CK 2 (91)	CP 43 (121.00), CP 48 (109.00), CP 84 (110.00), CP 128 (166.00), CP 157 (112.00), CP 182 (116.00)
Yield (quintal/ha)	IK 1 (45.41), IK 2 (49.67) and CK (38.11)	CP 57 (58.81), CP 84 (59.03), CP 128 (64.77), CP 130 (76.81), CP 134 (66.31), CP 154 (60.85)
Number of seeds/fruit	IK 1 (29.43), IK 2 (28.43), CK 2 (28.57)	CP 130 (38.00), CP 104 (38.00), CP 21 (33.00), CP 23 (33.00), CP 84 (34.00), CP 86 (36.00)
100 seed wt. (g)	IK 1 (9.64), IK 2 (9.77), CK 2 (9.94)	CP 43 (15.36), CP 80 (13.72), CP 115 (17.57), CP 116 (12.20), CP 131 (15.20), CP 169 (13.90)

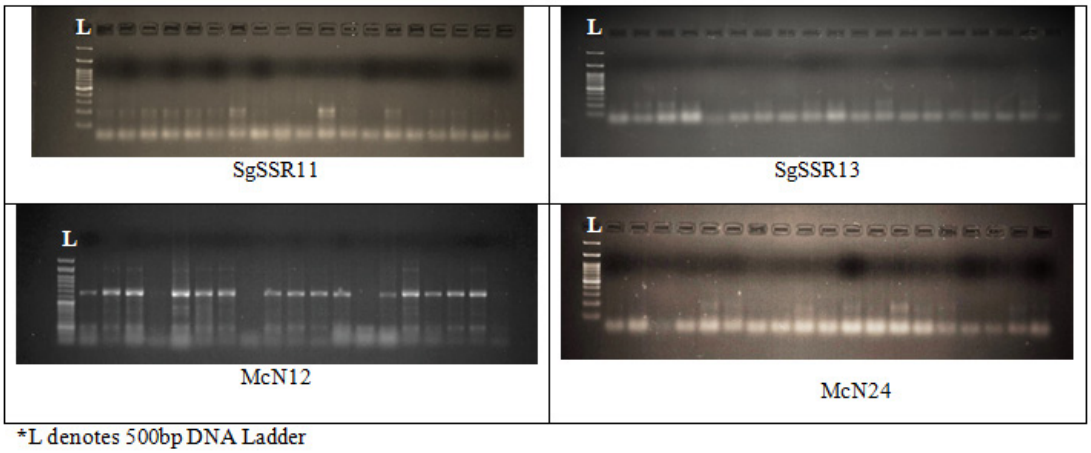

Figure 3: DNA profile of spine gourd genotypes obtained through microsatellite markers

in correlation and path analysis for yield attributing traits in bitter gourd. They observed from the path analysis that fruit number followed by fruit weight, days to first male flower anthesis, days to first female flower anthesis and fruit length exhibited maximum positive direct effect.

Genetic Variability Assessment

The genetic assessment of the lines was carried out using 20 microsatellite markers. Out of 20, 4 primers were found to be polymorphic. A total of 10 alleles were recorded using the 4 polymorphic microsatellite primers with an average of 2.5

Table 5: Correlation coefficient values

Trait	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
1	1															
2	0.597**	1														
3	0.457**	0.317**	1													
4	0.508**	0.364**	0.877**	1												
5	0.387**	0.261**	0.290**	0.237*	1											
6	0.626**	0.445**	0.501**	0.499**	0.366**	1										
7	-0.137NS	0.040NS	-0.136NS	-0.141NS	-0.088NS	-0.017NS	1									
8	-0.271**	-0.046NS	-0.243*	-0.254*	-0.149NS	-0.213*	0.775**	1								
9	-0.478**	-0.191NS	-0.414**	-0.421**	-0.349**	-0.491**	0.630**	0.858**	1							
10	0.248*	0.077NS	0.321**	0.341**	0.015NS	0.046NS	-0.090NS	-0.037NS	-0.042NS	1						
11	0.343**	0.151NS	0.415**	0.397**	0.396**	0.175NS	-0.266**	-0.229*	-0.357**	0.402**	1					
12	0.598**	0.392**	0.561**	0.631**	0.245*	0.514**	-0.014NS	-0.175NS	-0.313**	0.374**	0.434**	1				
13	0.732**	0.371**	0.560**	0.552**	0.553**	0.644**	-0.220*	-0.421**	-0.685**	0.056NS	0.367**	0.437**	1			
14	0.569**	0.267**	0.531**	0.521**	0.365**	0.476**	-0.177NS	-0.288**	-0.448**	0.505**	0.365**	0.533**	0.594**	1		
15	-0.094NS	-0.087NS	-0.011NS	-0.135NS	-0.047NS	-0.068NS	0.011NS	0.043NS	-0.001NS	-0.096NS	-0.106NS	-0.105NS	-0.074NS	0.109NS	1	
16	0.779**	0.428**	0.635**	0.704**	0.393**	0.636**	-0.128NS	-0.316**	-0.524**	0.301**	0.476**	0.891**	0.768**	0.638**	-0.119NS	1

* Significant at 1% ** significant at 5% NS- non significant

Here, characters are: 1-Days of 1st flowering, 2-number of 1st flowering nodes, 3-Leaf Length , 4-Leaf Width , 5-Pedicel length , 6- ovary length , 7- ovary Diameter , 8- Style Length , 9- Pistil tip length , 10-Fruit length , 11- fruit diameter , 12-Single fruit weight , 13-Number of fruit/plant, 14-Number of seed/fruit, 15-100 seed weight and 16-yield (quintal/ha)

Table 6: Direct and indirect effects of traits on yield

Trait	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
1	0.093	-0.027	-0.039	0.052	-0.015	-0.008	0.004	0.003	-0.033	0.002	0.006	0.395	0.360	-0.013	-0.001
2	0.055	-0.046	-0.027	0.037	-0.010	-0.006	-0.001	0.001	-0.013	0.001	0.003	0.259	0.183	-0.006	-0.001
3	0.042	-0.015	-0.086	0.089	-0.011	-0.007	0.004	0.003	-0.028	0.002	0.007	0.370	0.276	-0.012	0.000
4	0.047	-0.017	-0.075	0.102	-0.009	-0.006	0.004	0.003	-0.029	0.002	0.007	0.417	0.271	-0.012	-0.001
5	0.036	-0.012	-0.025	0.024	-0.038	-0.005	0.003	0.002	-0.024	0.000	0.007	0.162	0.272	-0.008	0.000
6	0.058	-0.020	-0.043	0.051	-0.014	-0.013	0.000	0.003	-0.034	0.000	0.003	0.339	0.317	-0.011	-0.001
7	-0.013	-0.002	0.012	-0.014	0.003	0.000	-0.028	-0.010	0.043	-0.001	-0.005	-0.009	-0.108	0.004	0.000
8	-0.025	0.002	0.021	-0.026	0.006	0.003	-0.022	-0.013	0.059	0.000	-0.004	-0.116	-0.207	0.007	0.000
9	-0.044	0.009	0.036	-0.043	0.013	0.006	-0.018	-0.011	0.068	0.000	-0.006	-0.207	-0.337	0.010	0.000
10	0.023	-0.004	-0.028	0.035	-0.001	-0.001	0.003	0.000	-0.003	0.007	0.007	0.247	0.027	-0.012	-0.001
11	0.032	-0.007	-0.036	0.040	-0.015	-0.002	0.008	0.003	-0.024	0.003	0.017	0.286	0.180	-0.008	-0.001
12	0.055	-0.018	-0.048	0.064	-0.009	-0.007	0.000	0.002	-0.021	0.002	0.007	0.660	0.215	-0.012	-0.001
13	0.068	-0.017	-0.048	0.056	-0.021	-0.008	0.006	0.005	-0.047	0.000	0.006	0.289	0.492	-0.014	-0.001
14	0.053	-0.012	-0.046	0.053	-0.014	-0.006	0.005	0.004	-0.031	0.003	0.006	0.352	0.292	-0.023	0.001
15	-0.009	0.004	0.001	-0.014	0.002	0.001	0.000	-0.001	0.000	-0.001	-0.002	-0.070	-0.036	-0.002	0.008

Residual factor - 0.0212 * Bold numbers are direct effects

Here, characters are: 1-Days of 1st flowering, 2-number of 1st flowering nodes, 3-Leaf Length (cm), 4-Leaf Width (cm), 5-Pedicel length (cm), 6- ovary length (mm), 7- ovary Diameter (mm), 8- Style Length (mm), 9- pistil tip length (mm), 10-Fruit length (cm), 11- fruit diameter (cm), 12-single fruit weight (g), 13-number of fruit/plant, 14-no. of seed/fruit, 15-100 seed weight (g)

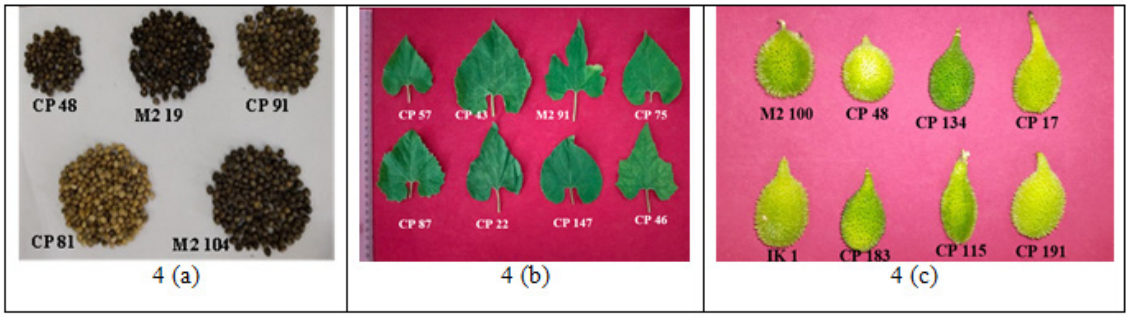

Figure 4: Variations observed in the population

alleles per primer. The number of alleles amplified for each primer ranged from 2 to 4. The polymorphic information content (PIC) for these primers ranged from 0.41859 to 0.598526. Table 7 shows the PIC values and number of alleles generated for the 4 polymorphic markers. The pattern of PCR images generated by the four polymorphic primers (SgSSR11, SgSSR13, McN12 and McN24) is given in Figure 3. Cluster analysis of the lines based on the molecular data was also generated. The cluster range was found to be from 0.510 to 1. The population was divided into 2 clusters (Cluster I and Cluster II) at a similarity 0.510, with 3 genotypes and 77 genotypes respectively. Cluster I is divided into 2 sub clusters (cluster IA and IIA) at a similarity 0.675 and cluster II is divided into 2 sub-clusters (cluster IIA and IIB) at a similarity 0.540. There were 18 genotype clusters with 100% similarity. Similar results were obtained by Alhariri *et al*. (2021) during their SSR marker-based analysis of btter gourd. They also analyzed the population structure of the genotypes.

Table 7: Size and frequency of alleles at four microsatellite loci in ninety-nine germplasm line of spine gourd

Primer	*No. of alleles*	*PIC Value*
SgSSR11	2	0.4745
SgSSR13	2	0.4186
McN12	4	0.5985
McN24	2	0.4753

Assessment of Population Structure

The information of population structure in mutant spine gourd germplasm based on allele frequency we used approaches as Pritchard *et al*. (2000). The analysis revealed the value of K ranged between 3-7. The optimal value is 4 (k=4), according to the likelihood provided by structure. According to the measure of K, which was discovered to be k = 4, the complete germplasm line can be classified into 4 clusters, subgroups, or subpopulations.

Conclusion

Based on the current study and analysis 10 lines were identified as very high-yielding and surpassing the checks with an enormous margin. The yield potential (q/ha) of the following lines: CP 57 (58.81), CP 61 (58.84), CP 84 (59.03), CP 100 (54.18), CP 104 (50.93), CP 128 (64.77), CP 130 (76.81), CP 134 (66.31), CP 145 (53.82) and CP 154 (60.85) was higher as against the checks: IK 1 (45.41), IK 2 (49.67) and CK (38.11). These lines can be used in selection or hybridization programs for further refinement of the genotypes and crop improvement. The morphological variation observed among the seeds, leaves shape and fruits shape among the mutant lines is given in Figure 4 (a), 4 (b) and 4 (c) respectively. Based on the desired characters, these lines can be selected for future breeding programs.

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सारांश

कांकोड़ा (*मोमोर्डिका डियोइका* रॉक्सब) की पहली उत्परिवर्ती पीढ़ी की आबादी पर एक अध्ययन किया गया था। प्रयोग में रूपात्मक और आणविक भिन्नता का मूल्यांकन करने के लिए कांकोड़ा की ९६ उत्परिवर्ती जर्मप्लाज्म लाइनों के साथ-साथ ३ जारी किस्मों को चेक के रूप में शामिल किया गया था। आणविक आनुवंशिक विश्लेषण द्वारा समर्थित रूपात्मक अनुवीक्षण की मदद से विशाल उत्परिवर्ती जननद्रव्य के बीच से कुछ उच्च उपज देने वाली लाइनें पाई गईं। रूपात्मक आंकड़े और विश्लेषण के आधार पर १० लाइनों की पहचान बहुत अधिक उपज देने वाली और भारी अंतर के साथ चेक से आगे निकलने वाली के रूप में की गई। इन पंक्तियों का उपयोग फसल सुधार के तहत चयन या संकरण कार्यक्रमों में किया जा सकता है। सहसंबंध अध्ययन से पता चला कि उपज का पहले फूल आने के दिनों (०.७७९), पत्ती की चौड़ाई (०.७०४), एक फल का वजन (०.८९१) और प्रति पौधे फलों की संख्या (०.७६८) के साथ अत्यधिक सकारात्मक संबंध था। पथ विश्लेषण से पता चला कि अधिकतम सकारात्मक प्रत्यक्ष प्रभाव एकल फल के वजन (०.६६०) और उसके बाद प्रति पौधे फलों की संख्या (०.४९२) था। जनसंख्या संरचना विश्लेषण से पता चला कि के का मान ३ से ७ के बीच है। इष्टतम मान ४ पाया गया (के=४) और इस प्रकार पूरी आबादी को इष्टतम रूप से ४ समूहों, उपसमूहों या उपआबादी में वर्गीकृत किया जा सकता है। रूपात्मक आंकड़े के माध्यम से समूह विश्लेषण से पता चला कि संपूर्ण आबादी समानता ०.७३ पर २ समूहों में विभाजित हुई हैं, जिसमें सीपी १०७ और सीपी १०५ (०.९६) के बीच सबसे अधिक समानता थी और एम २ ४ और सीपी १८२ (०.७३) के बीच सबसे कम समानता थी। आणविक डेटा के माध्यम से क्लस्टर विश्लेषण ने संपूर्ण आबादी को समानता ०.५१० पर २ समूहों में विभाजित किया। यहां १८ किस्म समूह पूरी तरह से (१००%) समान पाए गए और सबसे दूर की किसमे सीपी १४३ और एम २ १५१ थे। आणविक और रूपात्मक डेंड्रोग्राम का संयुक्त रूप से अध्ययन करने पर सीपी ५७ और सीपी १३० पंक्तियां उच्च उपज देने वाले और आनुवंशिक रूप से विविध दोनों पाए गए। अतः ये किसमे संकरण कार्यक्रमों के लिए चयनित किए जा सकते है। आणविक और रूपात्मक डेंड्रोग्राम के साथ साथ जनसंख्या संरचना विश्लेषण का संयुक्त रूप से अध्ययन करने पर सीपी ५७ और सीपी १३० पंक्तियां उच्च उपज देने वाले और आनुवंशिक रूप से विविध दोनों पाए गए। अतः ये किसमे संकरण कार्यक्रमों के लिए चयनित किए जा सकते है।