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Assessment of Fiber and Yield Related Traits in Mutant Population of Cotton

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ABSTRACT

The research work under consideration was conducted to study the genetic variability of the M_1 and M_2 generation of the cotton variety named Cyto-155. For M_1 generation, seed was previously collected from the Cotton Research Group Department of Plant Breeding and Genetics UAF and bombarded with gamma radiation at the Nuclear Institute of Agriculture and Biology (NIAB) in 2017-18. The doses given to M_1 seed were 20kR, 25kR, 30kR and 35kR. In 2018-19, the seed of M_1 generation was sown to raise the M_2 population in the research area University of Agriculture Faisalabad. Non-mutated seed along with mutated seed was sown in five lines for each treatment using a randomized complete block design (RCBD). At maturity data related to yield and fiber-related traits were collected. The analysis of variance for yield and fiber-related traits revealed significant differences in all the traits at different doses of gamma radiation in M_1 and M_2 generation. The basic statistics range, standard deviation and variance related to yield and fiber-related parameters of M_1 and M_2 generation also indicated the presence of variability. Seed cotton yield showed a positive correlation with plant height and negative correlation with ginning out turn and lint index in M_1 generation, while in M_2 a positive correlation between ginning out turn and fiber strength was found. For M_1 and M_2 generation, all four PCs displayed >1 eigen values and had maximum share to total variability. Plant height, sympodial branches and total number of nodes showed minimum differences as they were close to the origin whereas remaining all traits under study displayed maximum differences as they were at a greater distance from origin. For M_2 generation, Plant height and uniformity index showed minimum differences as they were close to the origin whereas remaining all traits under study displayed maximum differences as they were at greater distance from origin. These results show that radiation mutagenesis is an effective and feasible method to create variation which can further be exploited in future cotton breeding programs.

Key words: Mutation breeding, Fiber quality, Multivariate analysis

INTRODUCTION

Cotton is a vital crop in the economies of many nations, including Pakistan and its production has a considerable impact on the textile industry and economy (Onda and Mochida, 2016; Razzaq *et al*., 2021). The vast majority of the world's supply comes from tropical and subtropical areas. *Gossypium* is a genus comprised of 52 species, of which only four are cultivated economically. Although tetraploid varieties of Upland cotton (*Gossypium arboreum* L.), Egyptian cotton (*Gossypium barbadense* L.) and Egyptian cotton (*Gossypium hirsutum* L.) exist, tetraploid types of Asian cotton (*Gossypium arboreum* L.) and African cotton (*Gossypium herbacium* L.) are also available. Around 90% of global cotton production is sourced from upland cotton, with 8% from Egyptian cotton and 2% from other diploid kinds. Cotton's economic contribution in Pakistan cannot be underlined, according to latest research (Seyoum et al., 2018; Shim et al., 2018). Recent studies, such as the ones undertaken by Seyoum et al., show that Pakistan's cotton output per hectare is lower than that of other cotton-growing countries. There is a dearth of high-yielding lines that are resistant to harsh weather. Due to a paucity of germplasm, conventional

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breeding procedures have resulted in the development of only a few commercially successful types of cotton (Herring *et al*., 2004).

Without the assistance of genetic segregation or recombination, the genetic information of an organism can change swiftly and inheritably in response to chemical, physical, or biological events. This process is called mutagenesis (Roychowdhury *et al.*, 2013). Three distinct types of mutagenesis are employed during the mutation breeding process. Genetic transformation, as well as the insertion and activation of T-DNA, can result in DNA mutations, and site-directed mutagenesis is the process of introducing an anomaly in the DNA molecular structure at a specific point on the DNA molecule. The introduction of DNA into a cell results in mutations (Shu *et al.*, 2012; Shu, 2009). Plant breeding is necessary for agricultural success, as desired features require genetic diversity (Novak and Brunner, 1992). In comparison, a large number of mutant alleles provides genetic variety for crop breeding and functional studies on the targeted gene. A good outcome of mutation breeding is contingent upon the procedure's success, which entails two crucial steps: mutant screening and confirmation (Shu *et al*., 2012). Instead of picking individuals based on a narrow set of selection criteria such as early blossoming or disease resistance, the method of mutant screening chooses individuals from a significantly broader population of mutations (for example). Through the application of selective breeding techniques, a huge number of "putative mutants" and "false mutants" have been produced. Numerous samples are typically analyzed to identify probable mutations throughout the mutation confirmation technique. This technique has been used to demonstrate the invalidity of a large number of previously reported mutations. While single-base changes can affect protein production, crop improvement mutations are more prevalent. Any technique involving mutant breeding requires the execution of a predetermined series of actions. The efficacy with which desirable variant mutants can be selected in the second (M_2) or third (M_3) generation of the process is a factor impacting the efficiency of mutation breeding in compared to other breeding processes.

Before starting with a thorough inspection and analysis, mutant breeding experts first reduce the number of mutations present in mutagenized seeds of the first (M_1) plant generation to a manageable level (Roychowdhury *et al*., 2013). It is critical to know the number of mutants you intend to create in the initial generation of trials to ensure long-term success in mutation breeding. If the population under investigation allows for a large number of mutation measures, a large number of mutation measurements should be performed. As a result, the breeder must constantly monitor the entire population for contamination. When it comes to the target gene, inheritance patterns have an effect on the population size. Utilize mutagens with a high mutation rate to maintain a reasonable population size for the M_1 generation (Roychowdhury *et al*., 2013). Mutant M_1 plants are heterozygous for one of two alleles and lack the other allele. This is because each mutation has a specific effect on a single allele during treatment. On the other hand, the probability of harboring a mutation on both alleles concurrently is governed by the mutation's frequency. This scenario is highly improbable. Because M_1 contains solely dominant mutations, there is currently no mechanism for assessing the expression of recessive mutants. In this setting, breeders should be vigilant for segregation-causing mutations in later generations. The plant breeder's efforts result in the production of homozygotes for dominant or recessive genes. Within the M_1 population, cross pollination should be avoided because it results in extra variation that is difficult to identify from the effects of mutational alterations (Roychowdhury *et al.*, 2011; Roychowdhury *et al*., 2013). The M_2 generation serves as a starting point for the screening and selection process. Screening/selection techniques, according to Roychowdhury *et al.*, 2011 can be classified into three broad categories (Roychowdhury *et al*., 2012). Visual and phenotypic approaches are also accessible in addition to physical and mechanical techniques. By utilizing appropriate screening technologies, which can be performed through physical or mechanical selection, seeds can be recognized based on their shape, size, weight, and density. Visual screening is the most effective and efficient technology available at the moment for identifying mutant characteristics. Visual/phenotypic selection can be used to choose a range of traits, including plant height and soil adaptability, as well as disease resistance, colour changes, early maturity, ion-shattering, and climatic adaptation. Physiological, biochemical, and chemical screening procedures, as well as physio-chemical screening, have been used to uncover mutants classified as "others." Once a promising trait of a mutant line is identified in its DNA, the seeds of the mutant line are multiplied to allow for longer field testing. This scenario will examine the mutant line, the mother cultivar, and a variety of distinct varieties of the plant. When comparing mutants to previously discovered species, the same principles should apply. Before a mutant can be declared economically viable, it must outperform the mother crop in field tests. To establish the economic feasibility of a potential mutant, a variety of features such as growth habit, structure, and yield components should be examined in a variety of conditions with varying water availability (including plant density and sowing dates) (Roychowdhury *et al*., 2013).

The following objectives were included in this study

1. Creation of genetic variability in a cotton variety through induced mutation.
2. Evaluation of cotton mutants for yield components and fiber-related traits.

MATERIALS AND METHODS

The research work under consideration was conducted in the research area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Various steps for this experiment are discussed below. The present research was conducted to grow the M_1 and M_2 generation of Cyto-155. For M_1 generation seed was previously collected from the Cotton Research Group Department of Plant Breeding and Genetics UAF and bombarded with gamma radiation at the Nuclear Institute of Agriculture and Biology (NIAB) in 2017-18. The doses given to M_1 seed were 20kR, 25kR, 30kR and 35kR. In 2018-2019, the seed of M_1 generation was sown to raise the M_2 population in the research area University of Agriculture Faisalabad. Non-mutated seed along with mutated seed was sown in five lines for each treatment

using randomized complete block design (RCBD). Row to row distance and plant to plant distance was maintained 75cm and 30cm respectively. At maturity data was collected for the following traits:

1) Plant height (cm)
2) Number of monopodial branches
3) Number of sympodial branches
4) First fruiting branch number
5) Total number of nodes
6) Height to node ratio
7) Weight after ginning (g)
8) Seeds per boll
9) GOT%
10) Number of Bolls per plant
11) Boll weight (g)
12) Seed index (g)
13) Lint Index (g)
14) Seed cotton yield (g)
15) Short fibers content (mm)
16) Upper half-mean length (UHML)
17) Fiber Strength (g/tex)
18) Uniformity Index (%)
19) Fiber fineness (µg/inch)
20) Fiber Maturity

Seed cotton yield was determined through digital weight balance for every plant. After that, all samples were ginned in the laboratory of Plant Breeding and Genetics, UAF by using a single roller electrical gin. Now lint weight was determined with the help of digital weight balance. Through this information GOT% was calculated by using the following formula.

$$\text{Ginning out turn (GOT \%)} = \frac{\text{Lint Weight} \times 100}{\text{Seed cotton weight}}$$

The ginning percentage does not give any idea about total fiber production. The lint index gives us the proportion of fiber in the seed cotton sample. For estimating the lint index following formula was used.

$$\text{Lint index} = \frac{\text{(Weight of 100 seeds x Ginning percent)}}{\text{(100 – Ginning percent)}}$$

Parameters related to fiber quality were measured with High Volume Instrument (HVI) available at the Department of Fiber and Textile Technology UAF.

RESULTS

Analysis of Variance and Basic Statistics

The analysis of variance for yield and fiber-related traits for 5 treatments is given in Table 1. It revealed significant differences in all the traits at different doses of gamma radiation in M_1 and M_2 generations. The basic statistics range, standard deviation and variance related to yield and fiber-related parameters of M_1 and M_2 generation are displayed in the Table 2. In M_1 generation, MB varied from 0.51 to 1.18 having standard deviation 0.16 and variance 0.03. For SB, range was 5.15 to 9.28, standard deviation was 0.92 and variance was 0.03. For PH, range was 89.87cm to 103.08 cm, standard deviation was 3.57and variance was 12.71. For FFBN, range was 8.82 to 12.32 cm, standard deviation was 1.10 and variance was 1.22. For TN, range was 38.16 to. 43.15, standard deviation was 1.46, and variance was 2.13. For HNR, range was 1.54 to 2.17, standard deviation was 0.17 and variance was 0.03.

Table 1: ANOVA of fiber and yield related traits for M_1 and M_2 generation

	MSS Rep		MSS (Gen)		MSE	
	M_1	M_2	M_1	M_2	M_1	M_2
BW	0.11	0.11	0.18	0.22	0.04	0.05
FFBN	0.22	0.21	0.95	0.96	0.15	0.15
HNR	0.09	0.07	0.04	0.04	0.01	0.008
MB	0.07	0.07	0.04	0.04	0.01	0.01
PH	33.16	33.15	19.07	19.07	5.49	5.48
SB	0.12	0.12	2.19	2.19	0.59	0.58
SCY	3.26	4.82	31.59	35.5	8.26	8.83
SI	0.04	0.04	0.24	0.23	0.05	0.05
TB	0.11	8.66	2.40	13.69	0.22	2.91
TN	5.15	1.87	3.32	2.49	0.98	0.59
GOT	7.83	7.83	5.59	5.59	1.37	1.37
Li	0.26	0.06	0.89	1.57	0.12	0.05
MAT	0.00	0.001	0.00	0.00009	0.00	0.00002
Mic	0.01	0.01	2.13	0.03	0.02	0.008
SF	0.01	0.005	0.17	0.17	0.04	0.03
SPB	2.22	2.21	14.29	14.29	3.99	3.99
STR	0.14	0.44	14.49	1.74	0.07	0.48
UHML	0.25	0.21	2.05	2.07	0.57	0.57
UI	2.19	2.19	14.82	14.81	2.99	2.99

Table 2: Basic statistics of fiber and yield related traits for M_1 and M_2 generation

	Min		Max		Std		Variance	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
MB	0.51	0.53	1.18	1.20	0.16	0.16	0.03	0.03
SB	5.15	6.13	9.28	10.26	0.92	0.92	0.85	0.85
PH	89.87	93.22	103.08	106.43	3.57	3.57	12.71	12.71
FFBN	8.82	9.80	12.32	13.43	1.10	1.13	1.22	1.27
TN	38.16	41.24	43.15	45.49	1.46	1.09	2.13	1.19
HNR	1.54	1.96	2.17	2.62	0.17	0.16	0.03	0.03
TB	6.85	8.19	17.00	18.34	2.19	2.48	4.79	6.14
SCY	26.87	29.21	39.97	43.20	3.52	3.72	12.38	13.81
BW	2.35	2.38	3.42	3.57	0.28	0.31	0.08	0.10
SI	6.34	6.46	7.47	7.59	0.30	0.30	0.09	0.09
GOT	32.11	33.56	38.29	39.74	1.81	1.81	3.29	3.29
LI	3.03	2.56	4.91	5.49	0.55	0.74	0.30	0.54
SPB	15.83	17.30	26.65	28.12	2.42	2.42	5.88	5.88
MIC	2.54	3.31	5.47	3.81	0.67	0.12	0.45	0.01
STR	25.61	26.61	30.02	29.76	1.23	0.86	1.50	0.75
MAT	0.77	0.80	0.82	0.85	0.02	0.02	0.00	0.00
UI	74.65	75.88	82.98	84.21	2.31	2.31	5.36	5.36
UHML	25.92	26.15	29.64	29.87	0.91	0.91	0.84	0.83
SF	7.38	7.61	8.08	8.31	0.25	0.25	0.06	0.06

For TB, range was 6.85 to 17, standard deviation was 2.19 and variance was 4.79. For SCY, range was 26.87 to 39.97, standard deviation was 3.52 and variance was 12.38. For BW, range was 2.35 to 3.42, standard deviation was 0.28 and variance was 0.08. For SI, range was 6.34 to 7.47 in M_1 generation. For GOT, range was 32.11 to 38.29 and standard deviation was 1.81. For LI, range was 3.03 to 4.91, for SPB, range was 15.83 to 26.65. For MAT, standard deviation was 0.02 and for UI was 2.31. For SF, range was 7.38 to 8.08, standard deviation was 0.25 and variance was 0.06.

In M_2 generation, MB varied from 0.53 to 1.20 having standard deviation 0.16 and variance 0.03. For SB, range was 6.13 to 10.26, standard deviation was 0.92 and variance was 0.03. For PH, range was 93.92 cm to 106.43 cm, standard deviation was 3.57and variance was 12.71. For FFBN, range was 9.80 to 13.43 cm, standard deviation was 1.13 and variance was 1.27. For TN, range was 41.24 to. 45.49, standard deviation was 1.09 and variance was 1.19.

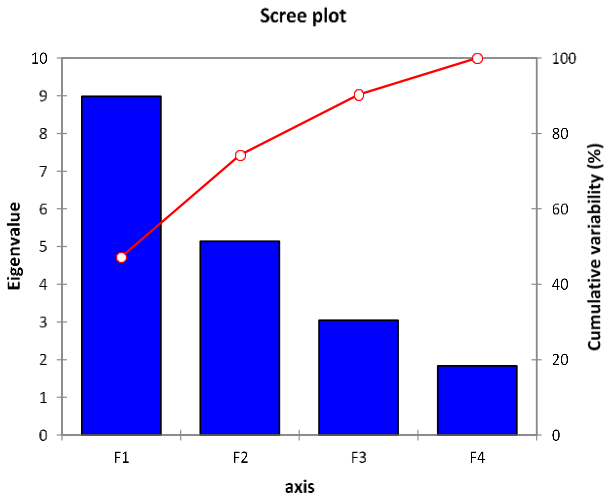

Fig. 1: Scree plot of M_1 generation.

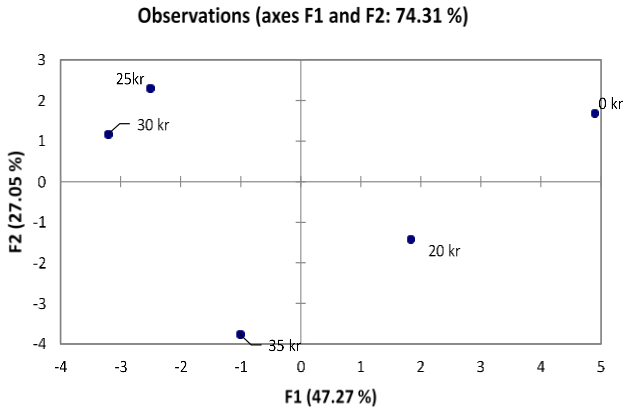

Fig. 2: Variation among doses in M_1 generation.

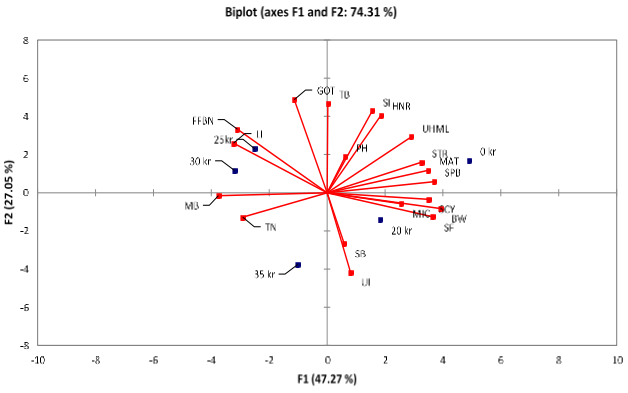

Fig. 3: Biplot graph of M_1 generation.

For HNR, range was 1.96 to 2.62, standard deviation was 0.16 and variance was 0.03. For TB, range was 8.19 to 18.34, standard deviation was 2.48 and variance was 6.14. For SCY, range was 29.21 to 43.20, standard deviation was 3.72 and variance was 13.81. For BW, range was 2.38 to 3.57 and standard deviation was 0.31. For SI, range was 6.46 to 7.59 in M_1 generation. For GOT, range was 33.56 to 38.29 and standard deviation was 1.81. For LI, range was 2.56 to 5.49, for SPB, range was 17.30 to 28.12. For MAT, standard deviation was 0.02 and for UI was 2.31. For SF, range was 7.61 to 8.31, standard deviation was 0.25 and variance was 0.06.

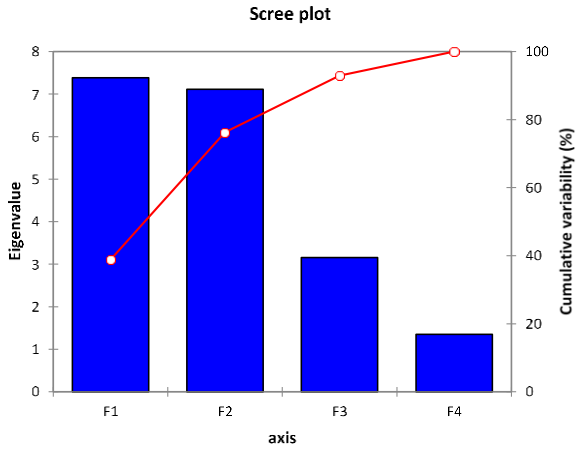

Fig. 4: Scree plot of M_2 generation.

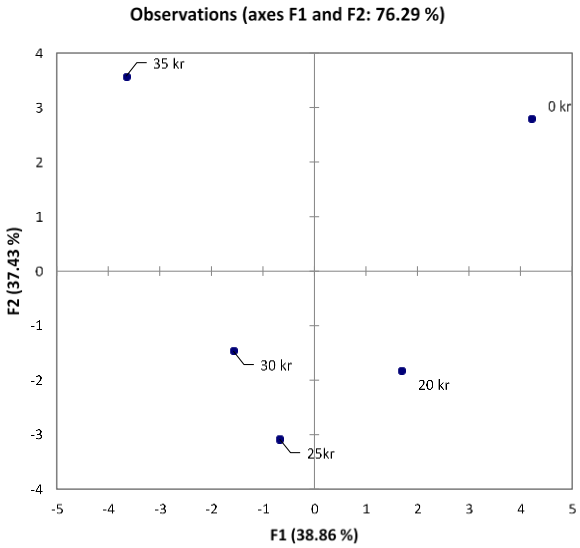

Fig. 5: Variation among doses in M_2 generation.

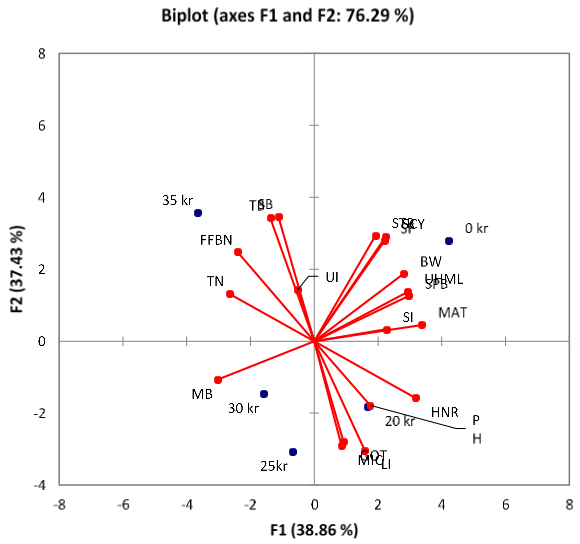

Fig. 6: Biplot graph of M_2 generation.

Correlation Analysis

The analysis of correlation on different yield-related traits and fiber quality traits performed for the M_1 population of cotton is illustrated in Table 3. Sympodial branches showed negative correlation with MAT.

Table 3: Correlation analysis of fiber and yield related traits in M_1 generation

M_1	SB	PH	FFBN	TN	HNR	TB	SCY	BW	SI	GOT	LI	SPB	MIC	STR	MAT	UI	UHML	SF
SB	0.05																	
PH	0.25	-0.30																
FFBN	0.29	0.47*	0.12															
TN	0.4	0.14	0.33	0.35														
HNR	0.04	-0.21	0.59**	0.11	-0.21													
TB	-0.12	0.57**	-0.10	0.55*	0.11	0.02												
SCY	-0.20	0.51*	0.07	0.13	0.14	0.21	0.18											
BW	-0.16	0.05	0.20	0.03	-0.04	0.41	0.07	0.531*										
SI	-0.16	-0.02	0.15	0.007	-0.13	0.38	-0.15	0.482*	-0.01									
GOT	0.28	-0.17	0.35	-0.16	-0.01	0.21	-0.4*	0.053	-0.39	0.5*								
LI	-0.01	-0.6**	0.49*	-0.48*	-0.19	0.46*	-0.9*	-0.211	0.033	0.03	0.33							
SPB	-0.29	-0.18	0.06	-0.21	-0.18	0.11	-0.09	0.27	0.53*	0.11	-0.1	0.07						
MIC	0.11	-0.44	0.34	0.019	0.08	0.36	-0.4*	-0.09	0.07	0.08	0.21	0.61**	-.02					
STR	0.05	0.26	0.20	0.306	0.30	0.15	0.08	0.69**	0.427	0.4*	-.02	-0.25	0.32	0.1				
MAT	-0.6*	-0.19	-0.21	-0.51*	-0.19	-0.37	-0.12	-0.06	-0.10	0.08	-.07	-0.06	0.01	-0.2	-0.13			
UI	-0.06	0.31	0.17	-0.11	0.31	-0.19	0.13	0.29	0.14	-0.3	-0.2	0.01	-0.1	-0.1	0.12	0.1		
UHML	-0.22	0.09	-0.05	-0.05	-0.4*	0.15	0.01	0.24	0.33	0.29	-0.2	-0.02	0.5*	-0.1	0.39	-.1	-0.17	
SF	-0.26	0.421	0.02	0.05	0.11	0.02	0.16	0.72**	0.46*	0.19	-0.1	-0.26	0.41	-0.1	0.6**	0.2	0.23	0.1

Table 4: Correlation analysis of fiber and yield related traits in M_2 generation

M_2	SB	PH	FFBN	TN	HNR	TB	SCY	BW	SI	GOT	LI	SPB	MIC	STR	MAT	UI	UHML	SF
SB	0.06																	
PH	0.25	-0.30																
FBN	0.29	0.47*	0.12															
TN	0.44	0.14	0.33	0.35														
HNR	0.04	-0.22	0.59**	0.12	-0.21													
TB	-0.12	0.5**	-0.10	0.55*	0.11	0.02												
SCY	-0.20	0.5*	0.08	0.14	0.14	0.22	0.18											
BW	-0.16	0.06	0.20	0.04	-0.04	0.42	0.07	0.531*										
SI	-0.17	-0.03	0.15	0.01	-0.13	0.39	-0.16	0.482*	-0.01									
GOT	0.28	-0.18	0.36	-0.16	-0.02	0.22	-0.5*	0.05	-0.39	0.5*								
LI	-0.02	-0.61	0.49*	-0.4*	-0.20	0.46*	-0.6*	-0.21	0.03	0.03	0.33							
SPB	-0.30	-0.18	0.07	-0.22	-0.18	0.11	-0.10	0.27	0.53*	0.11	-0.1	0.07						
MIC	0.11	-0.45	0.34	0.02	0.09	0.36	-0.4*	-0.10	0.08	0.08	0.21	0.6**	-.02					
STR	0.05	0.27	0.20	0.31	0.31	0.15	0.09	0.69**	0.43	0.4*	-0.1	-0.25	0.32	0.1				
MAT	-0.6*	-0.19	-0.21	-0.5*	-0.20	-0.38	-0.13	-0.06	-0.10	0.08	-0.1	-0.06	0.01	-0.2	-0.13			
UI	-0.06	0.31	0.17	-0.11	0.31	-0.19	0.14	0.29	0.14	-0.3	-0.2	0.01	-0.1	-0.1	0.12	0.1		
HML	-0.23	0.09	-0.05	-0.06	-0.4*	0.16	0.01	0.25	0.33	0.29	-0.2	-0.02	0.5*	-0.1	0.39	-0.1	-0.17	
SF	-0.27	0.42	0.03	0.05	0.12	0.03	0.16	0.72**	0.46*	0.19	-0.1	-0.26	0.41	-0.1	0.7**	0.22	0.23	0.15

The plant height was found positively correlated with FFBN, TB and SCY but it was negatively correlated with LI. The total number of nodes was found negatively correlated with MAT and LI. The total number of bolls was positively associated with TB. Boll weight was found positively correlated with seed index and STR The seed index had a positive correlation with SPB. Ginning out turn was found positively correlated with STR. There was found a positive association between lint index and seed index. Fiber maturity and short fiber content had a positive association. Fiber length and uniformity index were positively correlated. Seed cotton yield showed positive correlation with plant height and negative correlation GOT, LI and MIC.

The analysis of correlation on different yield-related traits and fiber quality traits performed for the M_2 population of cotton is illustrated in Table 4. Plant height was found positively correlated with the FBN, TB and SCY. The first fruiting branch number was positively associated with the HNR. The total number of nodes was found negatively associated with TB. The total number of bolls was positively associated with TN, PH and LI. Boll weight had a positive association with seed index, STR and SF. The seed index was positively correlated with SPB and SF. There was found a positive correlation between ginning out turn and STR. Lint index was found negatively associated with TN and SCY. Fiber fineness and short fiber content was found negatively correlated with each other. Fiber strength and HML was positively correlated with each other. Fiber length and short fiber content was negatively associated with each other.

Principal Component Analysis

Mean data of all the studied traits of M_1 generation was analyzed for PCA to study the genetic divergence by using XLSTAT. All four PCs displayed >1 eigen values and had maximum share to total variability. PC-I, II, III and IV had share of 47.26%, 27.04%, 16.02% and 9.65% to total variability respectively (Table). These four PCs imparted 100% to total variability among studied genotypes. The values of eigen vectors are displayed in Table 4. PC- I was mainly related to MB, FFBN, SCY, LI, SPB, STR and SF. PH, SB, TN and MIC showed minimum differences as they were close to the origin whereas remaining all traits under study displayed maximum differences as they were at greater distance from origin.

Mean data of all the studied traits of M_2 generation was also analyzed for PCA to study the genetic divergence by using XLSTAT. All four PCs displayed >1 eigenvalues and had maximum share to total variability. PC-I, II, III and IV

Table 5: Eigen values of M_1 generation

	F1	F2	F3	F4
Eigenvalue	8.9810	5.1387	3.0454	1.8348
Variability (%)	47.2683	27.0460	16.0286	9.6571
Cumulative %	47.2683	74.3144	90.3429	100.0000

Table 6: PCs of M_1 generation

	F1	F2	F3	F4
MB	-0.9398	-0.0288	0.1146	0.3205
SB	0.1473	-0.5047	0.7770	0.3463
PH	0.1557	0.3537	-0.7731	0.5029
FFBN	-0.7754	0.6300	-0.0396	0.0140
TN	-0.7286	-0.2423	-0.0356	0.6397
HNR	0.4649	0.7677	-0.3929	0.2004
TB	0.0055	0.8849	0.0786	0.4591
SCY	0.8755	-0.0655	0.4004	0.2626
BW	0.9798	-0.1576	-0.0983	0.0739
SI	0.3901	0.8110	0.4338	0.0439
GOT	-0.2840	0.9231	-0.1467	-0.2138
LI	-0.8091	0.4917	0.2878	0.1439
SPB	0.9231	0.1087	0.0739	-0.3614
MIC	0.6412	-0.1084	-0.7455	-0.1460
STR	0.8163	0.3029	0.4919	0.0010
MAT	0.8704	0.2218	-0.3723	0.2336
UI	0.2010	-0.7881	-0.3420	0.4706
UHML	0.7282	0.5536	0.3389	0.2201
SF	0.9120	-0.2391	0.1771	0.2824

Table 7: Eigen values of M_2 generation

	F1	F2	F3	F4
Eigenvalue	7.3831	7.1117	3.1544	1.3508
Variability (%)	38.8584	37.4299	16.6023	7.1093
Cumulative %	38.8584	76.2884	92.8907	100.0000

Table 8: PCs of M_2 generation

	F1	F2	F3	F4
MB	-0.8295	-0.2868	-0.1680	0.4489
SB	-0.3107	0.9358	-0.1155	0.1201
PH	0.4820	-0.4809	0.4545	0.5743
FFBN	-0.6610	0.6669	-0.3217	-0.1219
TN	-0.7312	0.3550	0.5373	0.2251
HNR	0.8687	-0.4233	-0.1023	0.2361
TB	-0.3794	0.9234	-0.0451	0.0379
SCY	0.6154	0.7841	-0.0472	0.0655
BW	0.7678	0.5034	0.3214	-0.2318
SI	0.6229	0.0896	-0.7496	0.2052
GOT	0.2503	-0.7493	-0.5976	0.1372
LI	0.4355	-0.8152	0.3760	0.0661
SPB	0.8133	0.3385	-0.0325	-0.4721
MIC	0.2344	-0.7827	0.4288	-0.3853
STR	0.5280	0.7907	0.0231	0.3091
MAT	0.9250	0.1214	0.3308	0.1420
UI	-0.1408	0.3854	0.8952	0.1742
UHML	0.8072	0.3707	-0.4123	0.2027
SF	0.6045	0.7514	0.2634	0.0224

had a share of 38.85%, 37.42%, 16.6%, and 7.1% to total variability, respectively (Table). These four PCs imparted 100% to total variability among studied genotypes. The values of eigenvectors are displayed in Table 7. PH and UI showed minimum differences as they were close to the origin whereas remaining all traits under study displayed maximum differences as they were at a greater distance from the origin.

DISCUSSION

For a successful breeding program, the first and foremost prerequisite is the presence of genetic variability (Zafar *et al*., 2021). If genetic variability is present in a population, effective selection is possible. Otherwise, the breeder will carry junk with him and it will be wastage of time and resources (Zafar *et al*., 2022). Genetic variability in the plant is key for the selection of plants with desirable characteristics (Charlesworth and Wright, 2001; Manan *et al*., 2022). More the genetic variability is present in a population more are the chances of effective selection. In the case of cotton where the almost 95% area is cultivated for *G. hirsutum*, the narrow genetic background is a problem. There are fewer genetic differences among the cultivated varieties of cotton (Sahar *et al*., 2021). It is the need of the hour to broaden the genetic makeup of our

cultivated verities (Van Becelaere *et al.*, 2005). Mutation is one of the effective and widely used techniques to create genetic variability (Shu, 2009). The X-rays, gamma rays, fast neutrons can be bombarded on the seed of cotton to create genetic variability in the cotton. In addition to these physical mutagens, there also exist some biological mutagen e.g., insertion of T-DNA and tagging of transposons (Griffiths *et al.*, 2000).

The analysis of variance for yield and fiber related traits revealed significant differences in all the traits at different doses of gamma radiation in M_1 and M_2 generation. Patel *et al.* (2014) and Méndez-Natera *et al.* (2012) also observed similar results. The basic statistics range, standard deviation and variance related to yield and fiber related parameters of M_1 and M_2 generation are displayed in the table 2. In M_1 generation, MB varied from 0.51 to 1.18, SB, range was 5.15 to 9.28, PH range was 89.87cm to 103.08 cm, FFBN range was 8.82 to 12.32 cm, TN range was 38.16 to. 43.15 HNR range was 1.54 to 2.17, TB range was 6.85 to 17, SCY, range was 26.87g to 39.97g. For BW, range was 2.35 to 3.42, standard deviation was 0.28 and variance was 0.08. For SI, range was 6.34 to 7.47 in M_1 generation. For GOT, range was 32.11 to 38.29 and standard deviation was 1.81. For LI, range was 3.03 to 4.91, for SPB, range was 15.83 to 26.65. For MAT, standard deviation was 0.02 and for UI was 2.31. For SF, range was 7.38 to 8.08, standard deviation was 0.25 and variance was 0.06. In M_2 generation, MB varied from 0.53 to 1.20. For SB, range was 6.13 to 10.26, standard deviation was 0.92 and variance was 0.03. For PH, range was 93.92 cm to 106.43 cm and for FFBN, range was 9.80 to 13.43 cm. For HNR, range was 1.96 to 2.62and for TB, range was 8.19 to 18.34, standard deviation was 2.48 and variance was 6.14. For SCY, range was 29.21 to 43.20. For BW, range was 2.38 to 3.57 and standard deviation was 0.31. For SI, range was 6.46 to 7.59 in M_1 generation. For GOT, range was 33.56 to 38.29 and standard deviation was 1.81. For LI, range was 2.56 to 5.49, for SPB, range was 17.30 to 28.12. For MAT, standard deviation was 0.02 and for UI was 2.31. For SF, range was 7.61 to 8.31, standard deviation was 0.25 and variance was 0.06. Patel *et al.* (2014) observed increase in boll weight from 1.35 g/boll for control to 1.67 and 1.84 g/boll for mutated plants at different doses. Fiber quality traits and the yield contributing traits the mutants were performing much better than that of control lines. Some lines which were selected for the fiber quality traits were also conferring the multiple benefits for pleiotropic beneficial genes.

The information about the relation of yield and its components possesses considerable importance for the selection of desirable plants. It is found that change in one character alters the behavior of another trait because the trait depends upon each other. In cotton, the yield is dependent upon several traits e.g., no. of bolls/plant, no. of sympodia and monopodia etc. (Méndez-Natera *et al.*, 2012). The analysis of correlation is a prerequisite for fruitful research in cotton. Yield is directly associated with some traits either positively or negatively. The number of sympodia was positively correlated with yield and the number of monopodia and plant height are usually negatively correlates to yield. Without correlation analysis, we cannot conclude about our research that how the component traits are contributing to yield (Salahuddin *et al.*, 2010).

Patel *et al.* (2014) and Méndez-Natera *et al.* (2012) also observed similar results that the analysis of correlation on different yield-related traits and fiber quality traits performed for the M_1 population of cotton which indicated that sympodial branches showed negative correlation with MAT. The plant height was found positively correlated with FFBN, TB and SCY but it was negatively correlated with LI. The total number of nodes was found negatively correlated with MAT and LI. The total number of bolls was positively associated with TB. Van Becelaere *et al.*, 2005 found that boll weight was positively correlated with seed index and STR The seed index had a positive correlation with SPB. Ginning out turn was found positively correlated with STR. Charlesworth and Wright, 2001 was found a positive association between lint index and seed index. Fiber maturity and short fiber content had a positive association. Fiber length and uniformity index were positively correlated. Seed cotton yield showed positive correlation with plant height and negative correlation GOT, LI and MIC.

For the M_2 population of cotton Plant height was found positively correlated with the FBN, TB and SCY. The first fruiting branch number was positively associated with the HNR. The total number of nodes was found negatively associated with TB. The total number of bolls was positively associated with TN, PH and LI. Boll weight had a positive association with seed index, STR and SF. The seed index was positively correlated with SPB and SF. There was found a positive correlation between ginning out turn and STR. Lint index was found negatively associated with TN and SCY. Fiber fineness and short fiber content was found negatively correlated with each other. Fiber strength and HML was positively correlated with each other. Fiber length and short fiber content was negatively associated with each other. Farooq *et al.* (2014) also found that Number of bolls per plant, plant height, boll weight, fiber length and strength, earliness index and GOT% were traits that were positively correlated with yield. Salahuddin *et al.* (2010) found that Number of sympodial branches per plant correlated positively with yield for all genotypes. Ashokkumar and Ravikesavan (2010) observed that Days to flowering initiation, number of sympodial branches, number of bolls, boll weight, seeds per boll, GOT%, lint index, seed index, and fiber fineness were positively correlated with seed cotton yield.

PCA is efficient mean of measuring variation present among genotypes for yield and its related traits. It is usually used to reduce the dimensionality of huge data sets. It converts data sets having large number of variables to smaller sets containing almost all the larger data set information. Smaller sets are easy to explore and analyze. So, PCA is a mean to decrease number of variables with the preservation of all the information. It demonstrates existing variation pattern that is helpful in selection of genetically diverse genotypes. It assisted in identification of genotypes with better yield and fiber traits that enhance seed cotton yield (Munir *et al.*, 2020). It provided information about factor loading and share of each attribute to total variability. For M_1 and M_2 generation, all four PCs displayed >1 eigen values and had maximum share to total variability. PH, SB, TN and MIC showed minimum differences as they were close to the origin whereas

remaining all traits under study displayed maximum differences as they were at greater distance from origin. For M_2 generation, PH and UI showed minimum differences as they were close to the origin whereas remaining all traits under study displayed maximum differences as they were at greater distance from origin.

Conclusion

For any breeding program, the genetic variation key to success is. The more the variation in the germplasm more will be the chances of success. The mutation is one of the efficient ways of creating genetic variability. According to recent research Pakistan's cotton output per hectare is less than that of other cotton-growing countries. There is a dearth of high-yielding types that are resistant to harsh weather. Due to a paucity of germplasm, conventional breeding procedures have resulted in the development of only a few commercially successful types of cotton. The results of our study showed that radiation mutagenesis is an effective and feasible method to create variation which can further be exploited in future cotton breeding programs

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