Evaluation of Morphological Attributes in Tea Progenies Arising from Gamma-Treated Seeds

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ABSTRACT

A key step in characterization of germplasm is the identification of phenotypic variation present in a given population. A study was carried out to determine the effect of different dosages of gamma rays (50 and 100Gy) on phenotypic variation using 21 standardized morphological descriptors of the UPOV Tea Test Guidelines. The trial comprised of open-pollinated seed stocks from six commercial tea cultivars namely TRFCA SFS150, TRFK 303/1199, EPK C12, GW Ejulu-L, TRFK 301/1 and TRFK 301/4 along with untreated controls. Data was collected for three seasons (dry, warm wet and cold wet) using five randomly selected plants from each treatment. Principle Component Analysis using 17 informative descriptors showed the first eight principal components accounted for 78% of the total variance, with 15 being highly informative. Cluster analysis further identified characters such as young shoot anthocyanin colouration at base of the petiole, leaf blade shape/color/length, shoot color/length, density of pubescence, plant vigour and density of branches as most discriminating descriptors resulting in four phenotypically well-defined groups. Most traits showed significant correlation, an indication that the traits could be used for indirect selection. The study provides a basis for rapid and early screening of base populations for identification of elite cultivars.

Keywords: *Camellia sinensis*, irradiation, mutation, germplasm, descriptors
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INTRODUCTION

Tea (*Camellia sinensis* L. (O) Kuntze) beverages are the second most consumed liquids after water.[1-3] In most of the tea producing countries, the crop has been a source of revenue and has contributed significantly to the local rural economies.[4] The tea industry has contributed to poverty reduction, infrastructural development, and environmental conservation through enhanced water infiltration, reduced surface erosion and mitigation of global warming through carbon sequestration.[5] Plant genetic resources are finite and vulnerable to losses due to introduction of new crop varieties in agriculture, growing urbanization, natural hazards and climatic change.[6] Therefore, evaluation, characterization and screening of genetic resources are considered key priorities in breeding programs since such information from such initiatives is crucial in choosing material for the incorporation into breeding activities.[7]

The significance of utilizing genetic resources in breeding programs to enhance crop genetic potential has been well recognized.[8-10] However, the accessibility of germplasm depends largely on available information on characterization and evaluation. A number of studies to evaluate tea diversity have been conducted using morphological markers,[11-13] biochemical markers,[14-16] digital markers[17] and molecular markers.[13,18-24] Morphological traits are useful descriptors for preliminary characterization of genetic resources since they are cost effective as compared to other markers.[25] A key step for proper characterization of germplasm is to identify the phenotypic variation present in the given germplasm. In order to achieve this objective, the germplasm accessions need to be characterized using a standard set of descriptors such as the International Plant Genetic Resources Institute (IPGRI) published in 1997 and the Union for Protection of Plant Varieties (UPOV) that was later published in 2008. Information gathered can be used to understand patterns of genetic variation existing in crop species,[26,27] identify accessions with high genetic variability, and determine genetic relatedness among accessions.[28] In tea, morphological characters have been utilized to examine genetic diversity,[29,30] variation,[31,32] phylogeny and grouping.[33,34] An earlier

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study by Visser[35] showed that a combination of slight pigmentation (anthocyanin) in the presence of pubescence influences tea quality. Further, Owuor and Obanda[36] suggested the possibility of using morphological features and various chemical constituents in selecting for quality of tea at single bush level.

Conventional breeding techniques take long to avail elite tea varieties and often fail to provide desirable results for a combination of majority of desired traits. Consequently, alternative approaches such as mutation breeding are required since induced mutations effectively broadened genetic variability in cultivated crops.[41,42] Gamma irradiation is one of the main physical mutagens in mutation breeding which can induce either beneficial or deleterious effects on the chromosomes of crops.[37,38] Gamma rays belong to ionizing radiation which interact at molecular level to produce free radicals in cells. These radicals can damage or modify important components of plant cells and are reported to differentially affect the morphology, anatomy, biochemistry and physiology of plants depending on level of irradiation.[39,40] Mutation breeding has been recognized as a valuable supplement to conventional breeding in crop improvement since it exposes seeds or other parts of plants to chemical or physical mutagens changing the genetic composition of a cell.[41-44] Unlike conventional breeding procedures which

involve the production of new genetic combinations through gene assortment and segregation from already existing parental genes, mutation breeding induces exclusively new gene combinations that are evaluated in order to identify elite genotypes.[45] The mutants are thereafter subjected to selection in order to identify useful genotypes, an approach used in crop improvement to raise superior traits affecting plant size, flowering time and fruit ripening, fruit color, self-compatibility, self-thinning and resistance to pathogens[45]. The utilization of induced mutations for the improvement of crop plants has generated several mutants which have been used directly as new cultivars.[44, 46] Few studies have attempted to analyze effects of irradiation on the morphology of tea. Similarly, there have been few attempts to evaluate relationships between the individual morphological characters. The objective of the present study was to assess the stability of morphological characters for tea mutants with a view to explore their use as a measure of genetic diversity.

METHODOLOGY

An experiment to determine the effects of gamma irradiation at varying doses of open-pollinated seed stocks from six commercial cultivars TRFCA SFS150, TRFK 303/1199, EPK C12, GW Ejulu-l, TRFK 301/1 and TRFK301/4 was carried out at Timbilil Estate, Kenya Agricultural Research Organization (KALRO)-Tea Research Institute (0° 22′S, 35° 21′E, altitude 2178m above mean sea level), Kericho County, Kenya. This region is characterized by well-drained volcanic acidic soils of pH 4.0 – 5.8, well distributed annual rainfall of above 1200 mm and temperature range of 13–28°C[47] (Anon, 2002). The tea seeds were treated with four levels of gamma radiation i.e. 50, 100, 150 and 200Gy at the Biotechnology Research Institute of KALRO. Seedlings arising from 150 and 200Gy did not survive and hence were discarded from the successive evaluations. Only one seedling survived at 100Gy across all the stocks. Seedlings were raised in the TRI nursery before they were transplanted to the field (12°C) in form of a progeny trial.

EXPERIMENTAL DESIGN

Seventy two progenies comprising gamma treated open-pollinated seed stocks from the six commercial cultivars and untreated controls were planted as hedge at the recommended spacing of 1.22 x 0.61m. The parents and commercial cultivars were included as checks. Data was collected for three seasons (dry-December to March, warm wet- August to November and cold wet- April to July). Five randomly selected bushes from each treatment (0Gy, 50Gy) and one bush from 100Gy across the six stocks were used to record observations on morphological characters for evaluation of their phenotypic traits. Readings were taken in replicates and means across the three seasons used to curb biasness linked to plasticity of the morphological traits. Guidelines of the International Union for Protection of New Varieties of Plant (UPOV)[48] for the Conduct of Tests for Distinctness, Uniformity and Stability in Tea were used. Young tea shoot colour, immature leaf colour and mature leaf colour descriptors were measured using the Royal Horticultural Society (RHS) Colour Chart.[49] Twenty one tea descriptors were used to evaluate morphological traits among the progenies including their clonal controls. Quantitative tea descriptors like leaf length, width of third leaf and internode length were determined using a ruler. Presence and density of pubescence of the bud was observed by use of hand lens. All other qualitative traits were determined as per the UPOV 2008 guideline. Four characters found to be non-informative were eliminated from the analyses. Thus, 17 informative characters were used in statistical analysis (Table 1).

STATISTICAL ANALYSIS

Multivariate statistical techniques such as principal component analysis and cluster analysis are commonly used methods for characterization and analysis of genetic diversity for perennial crops such as tea.[31,50]

Principal Component Analysis (PCA)

In order to explore the pattern of variations in the measured characters and to determine the most informative characters for distinguishing the mutants, PCA was carried out using mean values of morphological observations across three seasons using Genstat software version 15.1.[51]

Cluster Analysis

The data for the evaluated populations was grouped by cluster analysis using the unweighted pair group method analysis (UPGMA) based on the similarity matrix of Euclidean distances of the morphological data. To trace the relationship among the progenies, a dendrogram, was generated using cluster analysis on first 8 principal coordinates (PCs). The statistical analyses were performed using Genstat software version 15.1.[51]

Frequency Distribution

The frequency distribution and the number of phenotypic classes were used to compute the Shannon-Index of Diversity (H')

Shannon-Index of Diversity (H')

Shannon-Index of Diversity (H') was used as a measure of phenotypic diversity of each trait as per the formula described by Spellerberg and Fedor.[52]

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

Where, S is the total number of species in the community, p_i is the proportion of total number of individuals (genotypes) in the ith class and n is the number of phenotypic classes. Each H' value was normalized by dividing it by its maximum value (log= n), which ensured that all scaled H' values were in the range 0 to 1.

RESULTS AND DISCUSSION

Principal Component Analysis (PCA)

Eigen values were generated from correlation matrix obtained by PCA using the means of 17 morphological descriptors scored for 72 test progenies and controls (Table 2). The values of the principle components (PC) indicated that the first 8 PCs accounted for 78 % of the total variation present in the cultivars that were retained for further analysis. Only few descriptor traits contributed significantly towards deciding the position of each PC (Table 3). The main contributor descriptors for each PC based on vector loadings are listed in Table 4. The first PC which accounted for 21% of the total variation was predominantly associated with the following descriptors; young shoot anthocyanin coloration at base of the petiole, leaf blade intensity of green color, leaf blade shape of apex and young shoot color of second leaf at 'two and bud' stage. The second PC which accounted for 13 % of the total variation was associated with leaf blade shape, leaf blade width and plant vigor. Density of branches, leaf blade shape and width, plant type and vigour were most important in reflecting the variation patterns associated with the third PC, which accounted for 11% of the total variation. Sinha and Mishra[61] was chosen to determine the cutoff

Table 1: Plant morphological descriptors of *Camellia* spp. used for diversity analysis (UPOV, 2008).

No.	Plant descriptor	Range /variation	Data type
1.	Plant vigor	Weak: 3; Medium: 5; Strong: 7	Quantitative
2.	Plant type	Shrubs:1; Semi-arbor: 3; Arbor: 5	Quantitative
3.	Plant growth habit	Upright:1; Semi-upright: 3; Spreading: 5	Quantitative
4.	Plant density of branches	Sparse: 3; Medium: 5; Dense: 7	Quantitative
5.	Young shoot density pubescence of bud	Sparse: 3; Medium: 5; Dense: 7	Quantitative
6.*	Leaf blade attitude	Upwards: 1; Outwards: 3; Downwards: 5	Quantitative
7.	Leaf blade shape	Very narrow elliptic: 1; Narrow elliptic: 2; Medium elliptic: 3; Broad elliptic: 4	Quantitative
8.	Leaf blade intensity of green color	Light: 3; Medium: 5; Dark: 7	Quantitative
9.*	Leaf blade shape of cross section	Folded upwards: 1; Flat: 2; Recurved: 3	Quantitative
10.	Leaf blade texture of upper surface	Smooth or weakly rugose: 1; Moderately rugose: 2; Strongly rugose:3	Quantitative
11.	Leaf blade undulation of margin	Absent or weak:1; Medium: 2; Strong:3	Quantitative
12.	Leaf blade serration of margin	Weak: 3; Medium: 5; Strong: 7	Quantitative
13.	Young shoot length of 'three and a bud'	Short: 3; Medium: 5; Long: 7	Quantitative
14.	Leaf blade length	Short: 3; Medium:5; Long: 7	Quantitative
15.	Leaf blade width	Narrow: 3; Medium: 5; Broad: 7	Quantitative
16.*	Plant branch zigzagging	Absent: 1; Present: 9	Qualitative
17.*	Young shoot pubescence of bud	Absent: 1; present: 9	Qualitative
18.	Young shoot anthocyanin coloration at base of petiole	Absent: 1; Present: 9	Qualitative
19.	Young shoot color of second leaf at 'two and a bud' stage	Whitish: 1; Yellow green: 2; Light green: 3; Medium green: 4; Purple green: 5;	Pseudo-Quantitative
20.	Leaf blade shape of apex	Obtuse: 1; Acute: 2; Acuminate: 3	Pseudo-Quantitative
21.	Leaf blade shape of base	Acute: 1; Obtuse: 2; Truncate: 3	Pseudo-Quantitative

Key: * Designates uninformative traits

Table 2. Eigen values of the correlation matrix obtained from the principle component analysis of 17 morphological descriptors.

PC	Eigen value	Difference	Proportion	Cumulative	Cumulative %
1	3.633	1.393	214	214	21.4
2	2.240	0.438	132	345	34.5
3	1.802	0.470	106	451	45.1
4	1.332	0.015	78	530	53.0
5	1.317	0.263	77	607	60.7
6	1.054	0.028	62	669	66.9
7	1.026	0.156	60	730	73.0
8	0.870	0.170	51	781	78.1
9	0.700	0.042	41	822	82.2
10	0.658	0.051	39	861	86.1
11	0.607	0.095	36	896	89.6
12	0.512	0.049	30	926	92.6
13	0.463	0.201	27	954	95.4
14	0.262	0.010	15	969	96.9
15	0.252	0.111	15	984	98.4
16	0.141	0.009	8	992	99.2
17	0.132		8	1000	100.0

Table 3. Eigen vectors for first eight PCs of the 17 morphological descriptors

Descriptor	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
1	0.3561	0.21342	-0.009	-0.3062	0.01051	-0.1802	-0.1349	0.0074
2	-0.2761	0.26187	0.3639	0.17582	0.1114	-0.3097	-0.2102	0.18293
3	0.13637	0.15903	0.10756	-0.2065	-0.528	-0.1893	0.14225	0.30116
4	-0.2525	0.28099	0.25201	-0.1489	-0.0782	0.21945	0.15901	-0.1789
5	0.3614	0.18053	0.11753	-0.2964	0.26151	0.04146	0.1925	0.02362
6	0.27955	0.15768	-0.1742	0.457	0.02492	0.16341	-0.1156	-0.1031
7	-0.0668	0.37605	-0.389	0.08492	0.23668	-0.0246	0.27087	0.21569
8	-0.2983	0.11294	-0.1492	-0.1912	0.1139	0.21554	0.26915	-0.078
9	0.04314	0.24311	-0.1883	0.05236	-0.602	-0.184	-0.0083	0.05819
10	0.01659	0.4743	-0.3034	0.10538	-0.0602	0.31952	0.09144	-0.0308
11	-0.0377	0.19884	0.4092	-0.1152	-0.1956	0.3595	-0.0529	-0.452
12	-0.22	0.3859	0.33731	0.19137	0.19599	-0.2087	0.0258	0.21611
13	0.3586	0.02027	0.23801	0.149	-0.1127	0.10272	0.18725	-0.0264
14	-0.0069	0.20224	-0.1277	-0.1959	0.03063	0.26148	-0.8	0.14365
15	0.0776	-0.1688	0.2276	0.02888	-0.0211	0.5512	0.08767	0.6993
16	0.4049	0.15954	0.11205	-0.2071	0.32773	-0.138	0.00836	-0.0349
17	0.24873	0.04716	0.16834	0.5497	-0.0004	0.04095	-0.0338	-0.113

Key: 1 Young shoot: anthocyanin coloration at base of the petiole
2. Density Of Branches
3. density of pubescence of bud
4. Plant: Growth Habit
5. Leaf blade: intensity of green color
6. Leaf blade: length

7. Leaf blade: shape
8. Leaf blade: shape of base
9. Leaf blade: serration of margin
10. Leaf blade: width
11. Plant: Type
12. Plant: Vigor
13. Leaf blade: shape of apex

14. Leaf blade: texture of upper surface
15. Leaf blade: undulation of margin
16. Young shoot: color of second leaf at 'two and bud' stage
17. Young shoot: length of 'three and a bud'

NOTE: Bolded values represent variables which made significant contribution to total variance. Sinha and Mishra [61] was selected to validate the cut-off limit for the coefficients of considerable vectors. According to this criterion, coefficients greater than 0.3 (regardless the direction positive or negative) have effects large enough to be considered important in the overall variation observed.

Table 4: Main contributor descriptors for each principal component (PC) based on vector loadings

PC	Main Descriptors
1	Young shoot: anthocyanin coloration at base of the petiole, Leaf blade: intensity of green color, Leaf blade: shape of apex and Young shoot: color of second leaf at 'two and bud' stage
2	Leaf blade: shape. Leaf blade: width and Plant: Vigor
3	Density Of Branches, Leaf blade: shape, Leaf blade: width, Plant: Type and Plant: Vigor
4	Young shoot: anthocyanin coloration at base of the petiole, Leaf blade: length and Young shoot: length of 'three and a bud'
5	Density of pubescence of bud, Leaf blade: serration of margin and Young shoot: color of second leaf at 'two and bud' stage
6	Leaf blade: width, Plant: Type and Leaf blade: undulation of margin
7	Leaf blade: texture of upper surface
8	Density of pubescence of bud, . Plant: Type and Leaf blade: undulation of margin

limit for the coefficients of the proper vectors where coefficients greater than 0.3 (regardless the direction positive or negative) as having important effects on the overall variation observed in the present study. The fourth and fifth PCs accounted for 8% each and were associated with young shoot anthocyanin coloration at base of the petiole, leaf blade length and young shoot length of 'three and a bud, density of pubescence of bud, leaf blade serration of margin, young shoot color of second leaf at 'two and bud' stage respectively. The remaining 3 PCs, which accounted for 17% of the total variation, were mainly associated with descriptors such as leaf blade width, plant type, leaf blade undulation of margin, leaf blade texture of upper surface and density of pubescence of bud. It was noted that only 15 of the 17 descriptors scored contributed significantly to the total variation present in the germplasm collection. Key among the contributor descriptors for each PC with agronomic importance were; anthocyanin coloration at base of the petiole, intensity of green color, color of second leaf and density of pubescence of bud. Previous studies have implicated these descriptors to be the maximum contributors for morphological variation[32]. Pubescence is an important trait in selection of high quality orthodox and white processed tea.[53-55]

Anthocyanin pigmentation is a chemical marker in the characterization of several tea cultivars with the advantage of being easily observable besides anthocyanins being potent

antioxidants.[56,57] Young shoot characters such as pigmentation in young leaves has been used for diversity analysis in tea.[58] Variation of pigment contents in tea has been attributed to environmental factors such as shade level, fertilizer application,[59] and cultivar differences.[60] Results from the current study indicated that gamma irradiation negatively influenced anthocyanin pigmentation. This reveals regulation of regulatory and/or structural genes that are involved in the flavonoid synthesis which controls catechins and anthocyanin biosynthesis might have gained or lost function following irradiation treatment. Most of the untreated progenies arising from clone TRFK 301/1 had high anthocyanin pigmentation.

Cluster Analysis

Based on the first 8 principle components of the PCA, a dendrogram was generated with 4 clusters (Figure 1). The cluster composition of different progenies revealed that 46% of the genotypes were in cluster 3 while 36% were in cluster 2. Accordingly, 10% and 8% of the genotypes were in clusters 1 and 4 respectively. Progenies from most 100Gy treatment were grouped in cluster 2 except those from TRFK 303/1199 and TRFK 301/4 that were in clusters 3 and 4, respectively. Similarly, all the control parents were in cluster 2. However, most of the 50Gy progenies were in cluster 3. No particular pattern was observed in the clusters based on gamma treatment. This probably indicate that other genetic factors such as resultant heterosis from the crossing, genetic recombination and gene assortment apart from gamma treatment could be responsible for the variation noted in the test tea progenies responsible for the variation noted in the test tea progenies.[62] Table 5 shows the key descriptors responsible for cluster divergence.

Figure 1: Dendrogram based on average linkage cluster analysis using 15 morphological descriptors of the 72 tea accessions

Table 5: The 7. Leaf blade: shape interpretation of the descriptors primarily responsible for cluster divergence

Cluster No.	Morphological descriptors mainly responsible for cluster divergence
Cluster 1	Young shoot: anthocyanin coloration at base of the petiole, Young shoot: length of 'three and a bud', Leaf blade: length, Leaf blade: intensity of green color
Cluster 2	Young shoot: color of second leaf at 'two and bud' stage, Density of pubescence of bud, Leaf blade: intensity of green color, Young shoot: anthocyanin coloration at base of the petiole
Cluster 3	Density of pubescence of bud, Leaf blade: shape of apex
Cluster 4	Plant: Vigor, Density of Branches, Young shoot: anthocyanin coloration at base of the petiole

Table 6: Frequency distribution and Shannon-Indices of diversity (H') of fifteen traits of gamma-treated progenies

No.	Traits/habit	Range /Variation	Frequency %	H'
1	Plant vigor	Weak: 3	12.5	0.170
		Medium: 5	61.1	
		Strong: 7	26.4	
2	Plant type	Shrubs:1	1.4	0.895
		Semi-arbor: 3	98.6	
		Arbor: 5	0.0	
3	Plant density of branches	Sparse: 3	22.2	0.167
		Medium: 5	62.5	
		Dense: 7	15.3	
4	Young shoot color of second leaf at 'two and a bud' stage	Whitish: 1	0.0	0.540
		Yellow green: 2	90.3	
		Light green: 3	0.0	
		Medium green: 4	0.0	
		Purple green: 5	9.7	
5	Young shoot density pubescence of bud	Sparse: 3	58.3	0.020
		Medium: 5	41.7	
		Dense: 7	0.0	
6	Young shoot anthocyanin coloration at base of petiole	Absent: 1	81.9	0.319
		Present: 9	18.1	
7	Young shoot length of 'three and a bud'	Short: 3	61.1	0.337
		Medium: 5	37.5	
		Long: 7	1.4	
8	Leaf blade length	Short: 3	51.4	0.273
		Medium:5	45.8	
		Long: 7	2.8	
9	Leaf blade width	Narrow: 3	59.7	0.331
		Medium: 5	38.9	
		Broad: 7	1.4	
10	Leaf blade shape of base	Very narrow elliptic: 1	23.6	0.154
		Narrow elliptic: 2	61.1	
		Medium elliptic: 3	15.3	
		Broad elliptic: 4	0.0	
11	Leaf blade intensity of green colour	Light: 3	91.7	0.691
		Medium: 5	5.6	
		Dark: 7	2.8	
12	Leaf blade texture of upper surface	Smooth or weakly rugose: 1	59.7	0.331
		Moderately rugose: 2	38.9	
		Strongly rugose:3	1.4	
13	Leaf blade shape of apex	Obtuse: 1	0.0	0.384
		Acute: 2	84.7	
		Acuminate: 3	15.3	
14	Leaf blade undulation of margin	Absent or weak:1	98.6	0.895
		Medium: 2	1.4	
		Strong:3	0.0	
15	Leaf blade serration of margin	Weak: 3	94.4	0.690
		Medium: 5	5.6	
		Strong: 7	0.0	

Table 7: Pearson similarity coefficient matrix utilizing fifteen key traits of the gamma-treated progenies

	Plant: Type	Density of Branches	Color of second leaf	Density of pubescence of bud	Anthocyanin coloration at base of the petiole	length of 'three and a bud'	Leaf blade: length	Leaf blade width	Leaf blade: shape of base	Intensity of green colour	Texture of upper surface	Leaf blade: shape of apex	Undulation of margin	Serration of margin
Plant: Vigor	.222*	.777**	-0.075	0.039	-0.167	-0.001	-0.088	0.168	.253*	-0.065	-0.139	-0.034	-0.027	-0.055
Plant: Type		0.182	0.039	0.1	0.056	0.092	-0.104	0.095	-.208*	0.034	0.095	0.05	0.014	0.029
Density Of Branches			-0.194	-0.089	-0.184	-0.043	-.224*	-0.04	0.058	-.258*	-0.084	-0.142	0.014	0.028
Color of second leaf				0.103	.699**	.288**	.289**	0.008	0.044	.743**	0.098	.382**	-0.039	-0.08
Density of pubescence of bud					.262*	0.05	0.03	0.081	-0.16	0.191	0.081	.268*	-0.1	.287**
Anthocyanin coloration at base of the petiole						0.123	.282**	0.11	0.005	.511**	.249*	.202*	-0.056	.201*
Length of 'three and a bud'							.441**	-0.004	-0.025	0.19	-0.107	.341**	0.137	0.046
Leaf blade: length								.318**	0.085	0.185	0.125	.304**	-0.11	0.104
Leaf blade: width									.497**	0.113	.231*	0.031	-0.095	.272*
Leaf blade: shape of base										0.095	-0.065	-0.193	-0.176	0.033
Intensity of green colour											0.113	.470**	-0.034	-0.069
Texture of upper surface												-0.043	-0.095	0.155
Leaf blade: shape of apex													-0.05	0.066
Undulation of margin														-0.029

Key: *, **: Correlation is significant at p ≤ 0.05, 0.01, respectively

Frequency Distribution of Traits

Frequency distribution of key morphological characters among the mutants is presented in Table 6. The plant vigour trait varied among the gamma treated progenies with 61.1% of the plants demonstrating medium vigour. The semi arbor plant type formed the greatest portion of the mutant plants with 98.6%. Plant density habits recorded from the mutant genotypes were sparse (22.2%), medium (62.5%) and dense type (15.3%). In addition, the distribution of mutant plants based on young shoot colour of second leaf at two and a bud stage were as follows; yellow green (90.3%) with the remaining 9.7% being purple green. Density pubescence of bud also varied with sparse (58.3%) and intermediate at 41.7%. Anthocyanin colouration at base of petiole was only present in 18.1% of the mutant plant studied. Most of the mutant plants (61.1%) had a short young shoot length at three and a bud stage. Leaf blade shape were mainly very narrow (23.6%), narrow (61.1%) and medium (15.3%) elliptic, respectively. Leaf blade intensity of green colour was mainly light (91.7%). The leaf blade texture of upper surface habit showed in the mutant accessions were smooth or weakly rugose (59.7%), moderately rugose (38.9%) and strongly rugose (1.4%) in that order. Leaf blade shape of apex observed in the tea mutants was acute (84.7%) and acuminate (15.3%). In addition, leaf blade undulation of margin was absent or weak (98.6%) and medium (1.4%). Leaf blade serration of margin observed in mutants was weak (94.4%) and medium (5.6%).

Shannon- Index of Diversity (H′)

The estimates of Shannon-Index of Diversity (H′) of the traits studied is presented in Table 6. High diversity values (below 0.500) were obtained in ten of the fifteen key traits examined. Considering all the traits, the minimum value of H′ was 0.895 for both plant type and leaf undulation of margin, while the maximum value was 0.020 for young shoot density pubescence of bud. A low H′ value, indicates diversity while high values indicate unbalanced frequency classes for an individual trait and lack of diversity for the trait. Traits such as leaf blade shape had a high value of H′ (0.154), followed by plant density of branches (0.167), plant vigour (0.170), leaf blade length (0.273), young shoot anthocyanin coloration at base of petiole (0.319), leaf blade texture of upper surface and leaf blade width (0.331) compared to plant type and leaf blade undulation of margin (0.895), leaf blade intensity of green colour (0.691), and leaf blade serration of margin (0.690) which had low values of H′. The diversity indices of the ten traits further suggest the presence of adequate dissimilarity among the evaluated genotypes that is a potential for tea improvement through selection.

Most traits showed significant ($p \leq 0.05$) correlation, indicating that some traits could be used to indirectly select for others in an improvement program (Table 7). For example, plant vigour was significantly ($p \leq 0.05$) positively correlated with plant type (r=0.222), density of branches (r=0.777; $p \leq 0.01$) and leaf blade: shape of base (r=0.253; $p \leq 0.05$). This is because plant vigour is based not only on the height of the plant but the number of stems, number of leaves and the branching present at time of scoring. Number of branches, bush area, shoots per bush and foliar phosphorus content are correlated with yield.[63] The arbor plant type may be generally preferred as it may simplify harvesting and cultural practices.

Plant type was negatively correlated with leaf blade shape of base (r=-0.208; $p \leq 0.05$), which indicates that accessions with semi arbor plant type may not necessarily produce narrow elliptic leaf blades. Density of branches had a negative relation ($p \leq 0.05$) with both leaf blade length (r=-0.224) and leaf blade intensity of green colour (r=-0.258). Young shoot colour at three and a bud stage positively correlated ($p \leq 0.01$) with anthocyanin colour at base of petiole (r=0.699), length of three and a bud (r=0.288), leaf blade length (r=0.289), leaf blade intensity of green colour (r=0.743) and leaf blade shape of apex (r=0.382). Density of pubescence of bud correlated significantly ($p \leq 0.01$) with leaf blade serration of margin (r=0.287), anthocyanin colour at base of petiole(r=0.262) and leaf blade shape of apex (r=0.268; $p \leq 0.05$). Interaction between anthocyanin colouration at base of petiole and leaf blade length, leaf blade intensity of green colour, texture of upper surface, leaf blade shape of apex and serration of margin was significant ($p \leq 0.05$). In addition, significant correlations ($p \leq 0.01$) were obtained between the values recorded in young shoot length of three and a bud against leaf blade length (r=0.441) and shape of apex (r=0.341). Similarly, leaf blade width highly correlated ($p \leq 0.01$) with leaf blade shape of base (r=0.497) and leaf blade length (r=0.318), texture of upper surface (r=0.231) and leaf blade serration of margin (r=0.272) at ($p \leq 0.05$). Indeed, leaf blade length is relative to leaf blade width while leaf size is an important physiological trait as it has a profound effect on productivity[64]. The interaction between leaf blade shape of apex and leaf blade intensity of green colour was also significant (r=0.470) at ($p \leq 0.01$). These results conform to those by Wachira[65] who recorded that all the shoot size traits were significantly and positively inter-correlated. Plant architecture, leaf form and size play a significant role in phytophagy.[66, 67]

CONCLUSION

This study described and estimated the extent of phenotypic variation present among the gamma-treated open pollinated progenies of tea. Data generated can be used to elucidate patterns of morphological variation existing in the mutants and to help identify progenies with key traits that could be used in early identification of cultivars for further evaluation and testing in the latter stages of tea improvement program. It is however recommended that the gamma treated progenies be analyzed using modern biochemical and molecular tools for a better understanding of the effect of the irradiation on tea plants.

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