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Chlorophyll categorization of gamma radiated tea (*Camellia sinensis* L.) population for conventional breeding objectives

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Abstract
Chlorophyll-a, chlorophyll-b, and carotenoids are vital components of the plant photosynthesis system and directly influence the yield and quality parameters of the tea plant. We measured chlorophyll-a, chlorophyll-b and carotenoids in radiated tea population. About 134 gamma-radiated induced tea populations were used for the estimation of photosynthetic pigment (Chl-a, Chl-b, and Car.) and their categorization as low, medium, and high basis. Contents of chl-a, chl-b, and car. varied significantly between tea planting materials. The correlations among the characters were significant and positive. This study is an attempt to indicate the available variation of the chlorophyll component of the radiated tea population and its categorization as low, medium and high. Based on the these preliminary data, our present study attempted to reveal various level of chlorophyll component in tea population and its application for developing the breeding strategies for the development of new tea varieties. It has also strongly suggested that induced radiation have ability to create variation in the photosynthetic component of tea planting materials.

Keywords: Breeding, chlorophyll, gamma radiation, tea, population

Introduction
Tea is a perennial plant, beverage with health benefits and economically sounds for the growing country as a cash crop. Baker *et al*, 2008 suggested that photosynthesis is the most important source of energy for plant growth and development. Natthiporn A. *et al*. 2019 [7] explained that chlorophyll is commonly found oil soluble pigments responsible for the green colour of plant and the structure of chlorophyll is closed ring.
Ying. L. *et al*. 2018 [6] explained that Chlorophyll variations were also found in various plant species and soil and climate factors had very small influence on chlorophyll. The economic part of the plant is 'Two leaves and a bud' which are generally plucked by the plucker's for the manufacturing of tea. Chlorophyll is a green photosynthetic pigment usually found in the green part of the plant and mainly stored in the chloroplast of the green leaf area of the plant which controls the photosynthetic process of the plant and thereby determines the productivity of the plant. Scheer H. *et al*., 2022 [8] advocated that the largest impact from natural photosynthesis is conceptual, the combination of reaction entre for charge separation with light harvesting complexes that are adaptable to the quality and quantity of absorbed light is key to natural photosynthesis.
Singh I.. D. 1980 [10] describes the importance of genetic diversity in the evolution of tea planting materials and use of non-conventional breeding techniques for increasing genetic diversity in tea. Singh S. K. *et al*. 2022 [11] reported that gamma radiation significantly influenced the agro-morphological growth parameter of the tea plant. Bera *et al*. 1997 [3] elaborated that chlorophyll contributes the 'blackness' of made tea which is considered to be one of the important criteria for in the commercial evaluation of tea. Chlorophyll high and low could affect the quality and quantity of the tea bushes. Atmaza P. I. M. *et al*. 2018 [1] Chlorophyll a content in fresh tea leaf varies from 1.39 to 5.39 mg.g-1 and chlorophyll-b ranges between 0.77 to 2.06 mg.g^{-1}. Chlorophyll content in tea increased gradually as the leaves matured and significantly increased the photosynthetic rate. Detailed investigation and categorization of the tea bushes in terms of Chlorophyll-a, Chlorophyll-b and Total carotenoids are very important and effective for the crossing programmed of any breeding trial in the long term.

The success in the breeding of any crop depends on the availability of diverse genetic variability. Chanu M. A. *et al*. 2020 [4] concluded that induced mutation can successfully employed to create genetic variability when it is preferred to improve definite trait in plant Singh I. D *et al*. 1979 [9] explained that the diverse planting materials could be evolved by the available natural variability or by the using of modern and cutting-edge technology for the creation of variation. Therefore, identification and estimation of those variable characters is highly important and always needs to be found in a continuous mode as compared to single chance.

Materials and Methods

The experimental materials comprised one hundred thirty four gamma radiated tea populations from which young two leaves and a bud part shoots were collected from the long term trail plot at New Botanical Area, Tocklai in the year 2022. All the selected radiated tea population was grown under identical environmental conditions and balanced nutrient management. Extraction was done with methyl alcohol following the method of Taylor S 1992 with some modification. 0.5 g of fresh shoots was grinded with 25 ml of methanol in a mortar under dark condition and filtered through a buchner funnel with the help of a vacuum pump. 2 ml of extracted solution was diluted to 10 ml with methanol before spectrophotometric observations were recorded in three different wavelengths, *viz*, 470 nm for Tc, 653 nm for chl-b and 666 nm for chl-a.

Statistical analysis: Analysis of variance (ANOVA) was performed by using an online version of O.P. Statistics. For each sample, all data were reported as the mean standard error (SE) with three replications for each season has also been computed to check for any significant differences among them.

Results and Discussion

Wide variations in chl-a, chl-b and Car. content was observed among the clones studied (Table-1). Differences between the clones in respect of the characters were statistically significant in most of the cases. Highly positive significant correlations (Table-2) were found among the three characters, which indicate that chlorophylls and carotenoids, being structural and functional components of the chloroplast, are correlated to each other as was suggested earlier (Hazarika and Mahanta, 1984) [5]. Based on the estimates they were grouped into three categories, i.e. low, medium and high (Table-2). Across all tea plant populations, variations were noticed and grouped belonging to the low, medium and high categories. 24 clones belonged to the low category of chl-a, whereas medium and high categories had 77 and 35 clones respectively (Table-2). In case of chl-b, high, medium and low category groups had 15, 71 and 50 clones respectively. For Car., 73 clones belonged to the medium category, 23 clones to the low and the remaining 40 clones to the high category. The present study reveals appropriate levels of chlorophyll variation have been found for the utilization in the further breeding experiment.

Table 1: Chlorophyll ‘a’ and Chlorophyll ‘b’ and Carotenoids content in St. 851.

Population of St. 851	Chlorophyll ‘a’ (mg g^{-1}FW)	Chlorophyll ‘b’ (mg g^{-1}FW)	Carotenoids (mg g^{-1}FW)
851/1	1.466	0.679	3.733
851/2	1.750	0.758	4.302
851/3	2.223	1.018	4.747
851/4	2.067	0.936	5.699
851/5	1.603	0.699	3.414
851/6	1.612	1.204	4.295
851/7	2.475	1.153	5.305
851/8	2.165	1.034	5.023
851/9	1.876	1.359	4.378
851/10	2.346	1.085	5.390
851/11	2.130	0.986	5.226
851/12	1.809	0.806	4.736
851/13	2.349	1.088	5.860
851/14	1.883	0.804	4.853
851/15	1.569	0.690	4.301
851/16	2.108	0.938	4.803
851/17	2.121	0.925	4.852
851/18	2.674	1.217	5.635
851/19	1.624	0.719	3.959
851/20	1.839	0.846	4.768
851/21	1.866	0.852	4.541
851/22	2.120	0.968	5.585
851/23	1.836	0.835	4.512
851/24	2.079	1.466	5.120
851/25	1.674	0.735	4.103
851/26	1.800	0.846	5.571
851/27	2.259	1.031	5.095
851/28	2.090	0.934	5.196
851/29	1.715	0.764	4.331
851/30	1.825	0.844	4.360
851/31	1.692	0.851	4.553
851/32	2.279	0.887	4.457
851/33	1.921	0.857	4.793
851/34	2.503	1.159	6.034
851/35	1.223	0.522	3.427

851/36	2.209	1.001	5.090
851/37	2.218	1.013	4.939
851/38	2.319	1.072	5.033
851/39	1.765	0.794	4.244
851/40	1.791	1.429	4.155
851/41	2.086	0.934	4.794
851/42	1.744	0.834	4.710
851/43	1.662	0.731	4.347
851/44	2.584	1.203	5.654
851/45	2.070	0.923	5.341
851/46	1.691	0.736	4.024
851/47	2.604	1.181	5.533
851/48	1.891	0.803	4.914
851/49	2.561	1.177	5.320
851/50	1.838	0.831	4.564
851/51	1.755	0.821	4.335
851/52	1.728	0.789	4.060
851/53	2.195	0.953	5.032
851/54	1.860	0.832	4.586
851/55	2.611	1.281	5.931
851/56	1.242	0.530	3.420
851/57	2.129	0.965	5.121
851/58	2.059	0.941	5.001
851/59	1.578	0.688	4.324
851/60	1.819	0.793	4.012
851/61	1.807	0.827	4.419
851/62	1.854	1.009	4.967
851/63	2.158	0.865	4.132
851/64	1.871	1.354	4.582
851/65	2.285	1.041	5.418
851/66	1.931	0.894	4.895
851/67	1.889	0.844	4.800
851/68	2.267	1.051	5.193
851/69	1.513	1.162	3.793
851/70	2.066	0.931	4.974
851/71	2.291	1.083	5.305
851/72	2.424	1.138	5.594
851/73	1.813	0.832	5.406
851/74	1.921	1.325	4.595
851/75	1.755	0.776	4.589
851/76	1.808	0.834	4.953
851/77	1.818	0.830	4.926
851/78	2.270	1.116	5.501
851/79	2.032	0.822	4.505
851/80	1.782	0.787	4.661
851/81	2.246	1.016	4.990
851/82	1.767	0.785	4.745
851/83	2.026	1.000	4.907
851/84	2.403	0.982	4.968
851/85	1.761	0.772	4.152
851/86	2.344	1.076	5.566
851/87	1.798	0.798	5.073
851/88	2.584	1.202	5.312
851/87	2.158	1.001	5.235
851/88	1.506	1.132	3.803
851/89	2.091	0.941	4.964
851/90	2.122	1.001	5.371
851/91	2.400	1.125	5.604
851/92	1.877	0.870	5.345
851/93	1.602	1.219	3.755
851/94	2.136	0.962	4.947
851/95	2.340	1.108	5.284
851/96	2.218	1.041	5.675
851/97	2.307	1.070	5.177
851/98	1.419	1.073	3.842
851/99	2.027	0.913	4.989
851/100	2.264	1.068	5.316

851/101	2.166	1.028	5.193
851/102	1.618	0.723	3.894
851/103	1.856	1.344	4.680
851/104	2.165	0.980	4.862
851/105	2.470	1.195	5.799
851/106	1.700	1.293	4.334
851/107	2.084	1.493	4.406
851/108	1.673	0.759	4.245
851/109	2.091	0.967	4.793
851/110	2.213	1.006	4.980
851/111	2.374	1.106	5.964
851/112	1.687	0.721	4.029
851/113	2.213	0.992	5.169
851/114	1.944	0.856	5.340
851/115	2.140	0.953	5.150
851/116	1.980	0.903	5.028
851/117	1.436	0.660	4.590
851/118	1.676	0.774	4.224
851/119	1.941	0.862	4.554
851/120	2.144	0.945	4.763
851/121	1.920	0.877	5.412
851/122	1.996	0.899	4.680
851/123	1.624	0.718	4.295
851/124	2.166	0.986	5.033
851/125	1.987	0.888	4.598
851/126	1.736	0.757	4.154
851/127	1.701	0.762	4.258
851/128	1.923	0.959	4.657
851/129	1.926	0.840	4.494
851/130	2.011	0.915	4.674
851/131	2.174	1.525	5.079
851/132	1.857	0.817	4.432
851/133	2.317	1.082	5.247
851/134	2.113	0.965	5.364
Mean	1.989	0.96	4.808
C.D at	0.356	NA	0.771

Method of categorization

A. For Chl-a

$$\frac{2.674\ (\text{Maximum}) - 1.223\ (\text{Minimum})}{3}$$

$$= \frac{1.451}{3} = 0.483$$

1.223 + 0.483 = 1.706; 1st category = 1.223 to 1.706
1.707 + 0.483 = 2.190; 2nd category = 1.707 to 2.190
2.191 + 0.483 = 2.674; 3rd category = 2.191 to 2.674

B. For Chl-b

$$\frac{1.526\ (\text{Maximum}) - 0.522\ (\text{Minimum})}{3}$$

$$= \frac{1.004}{3} = 0.334$$

0.522 + 0.334 = 0.856; 1st category =0.522 to 0.856
0.857 + 0.334 = 1.191; 2nd category = 0.857 to 1.191
1.192 + 0.334 = 1.526; 3rd category = 1.192 to 1.526

C. For Carotenoids

$$\frac{6.035 \text{ (Maximum)} - 3.414 \text{ (Minimum)}}{3}$$

$$= \frac{2.621}{3} = 0.873$$

3.414 + 0.873 = 4.287; 1st category = 3.414 to 4.287
4.288 + 0.873 = 5.161; 2nd category = 4.288 to 5.161
5.162 + 0.873 = 6.035; 3rd category = 5.162 to 6.035

Table 2: Categorization of clones according to Chl-a, Chl-b, and Carotenoids.

Category	Clones	No. of Clones
	Chlorophyll a	
Low (1.223 to 1.706)	851/1, 851/5, 851/6, 851/15, 851/19, 851/25, 851/31, 851/35, 851/43, 851/46, 851/56, 851/59, 851/69, 851/90, 851/95, 851/100, 851/104, 851/108, 851/110, 851/114, 851/119, 851/120, 851/125, 851/129.	24
Medium (1.707 to 2.190)	851/2, 851/4, 851/8, 851/9, 851/11, 851/12, 851/14, 851/16, 851/17, 851/20, 851/21, 851/22, 851/23, 851/24, 851/26, 851/28, 851/29, 851/30, 851/33, 851/39, 851/40, 851/41, 851/42, 851/45, 851/48, 851/50, 851/51, 851/52, 851/54, 851/57, 851/58, 851/60, 851/61, 851/62, 851/63, 851/64, 851/66, 851/67, 851/70, 851/73, 851/74, 851/75, 851/76, 851/77, 851/79, 851/80, 851/82, 851/83, 851/85, 851/87, 851/89, 851/91, 851/92, 851/94, 851/96, 851/101, 851/103, 851/105, 851/106, 851/109, 851/111, 851/116, 851/117, 851/118, 851/121, 851/122, 851/123, 851/124, 851/126, 851/127, 851/128, 851/130, 851/131, 851/132, 851/133, 851/134, 851/136.	77
High (2.191 to 2.674)	851/2, 851/7, 851/10, 851/13, 851/18, 851/27,851/32, 851/34, 851/36, 851/37, 851/38, 851/44, 851/47, 851/49, 851/53, 851/55, 851/65, 851/68, 851/71, 851/72, 851/78, 851/81, 851/84, 851/86, 851/88, 851/93, 851/97, 851/98, 851/99, 851/102, 851/107, 851/112, 851/113, 851/115, 851/135.	35
	Chlorophyll-b	
Low (0.522 to 0.682)	851/1, 851/2, 851/5, 851/12, 851/14, 851/15, 851/19, 851/20, 851/21, 851/23, 851/25, 851/26, 851/29, 851/30, 851/31, 851/35, 851/39, 851/42, 851/43, 851/46, 851/48, 851/50, 851/51, 851/52, 851/54, 851/56, 851/59, 851/60, 851/61, 851/67, 851/73, 851/75, 851/76, 851/77, 851/79, 851/80, 851/82, 851/85, 851/87, 851/104, 851/110, 851/114, 851/116, 851/119, 851/120, 851/125, 851/128, 851/129, 851/131, 851/134.	50
Medium (0.683 to 0.843)	851/3, 851/4, 851/7, 851/8, 851/10, 851/11, 851/13, 851/16, 851/17, 851/22, 851/27, 851/28, 851/32, 851/33, 851/34, 851/36, 851/37, 851/38, 851/41, 851/45, 851/47, 851/49, 851/53, 851/57, 851/58, 851/62, 851/63, 851/65, 851/66, 851/68, 851/69, 851/70, 851/71, 851/72, 851/78, 851/81, 851/83, 851/84, 851/86, 851/89, 851/90, 851/91, 851/92, 851/93, 851/94, 851/95, 851/96, 851/97, 851/98, 851/99, 851/100, 851/101, 851/102, 851/103, 851/106, 851/111, 851/112, 851/113, 851/115, 851/117, 851/118, 851/121, 851/122, 851/123, 851/124, 851/126, 851/127, 851/130, 851/132, 851/135, 851/136.	71
High (0.844 to 1.004)	851/6, 851/9, 851/18, 851/24, 851/40, 851/44, 851/55, 851/64, 851/74, 851/88, 851/105, 851/107, 851/108, 851/109, 851/133.	15
	Total Carotenoids	
Low (3.414 to 4.287)	851/1, 851/5, 851/8, 851/19, 851/25, 851/35, 851/39, 851/40, 851/52, 851/56, 851/60, 851/63, 851/69, 851/85, 851/90, 851/95, 851/100, 851/104, 851/110, 851/114, 851/120, 851/128, 851/129	23
Medium (4.288 to 5.161)	851/2, 851/3, 851/6, 851/9, 851/12, 851/14, 851/15, 851/16, 851/17, 851/20, 851/21, 851/23, 851/24, 851/27, 851/29, 851/30, 851/31, 851/32, 851/33, 851/36, 851/37, 851/38, 851/41, 851/42, 851/43, 851/46, 851/48, 851/50, 851/51, 851/53, 851/54, 851/57, 851/58, 851/59, 851/61, 851/62, 851/64, 851/66, 851/67, 851/70, 851/74, 851/75, 851/76, 851/77, 851/79, 851/81, 851/82, 851/83, 851/84, 851/87, 851/91, 851/96, 851/99, 851/101, 851/105, 851/106, 851/108, 851/109, 851/111, 851/112, 851/117, 851/118, 851/119, 851/121, 851/122, 851/124, 851/125, 851/126, 851/127, 851/130, 851/131, 851/132, 851/133, 851/134	73
High (5.162 to 6.035)	851/4, 851/7, 851/10, 851/11, 851/13, 851/18, 851/22, 851/26, 851/28, 851/34, 851/39, 851/40, 851/44, 851/45, 851/47, 851/49, 851/55, 851/65, 851/68, 851/71, 851/72, 851/73, 851/78, 851/86, 851/88, 851/89, 851/92, 851/93, 851/94, 851/97, 851/98, 851/102, 851/103, 851/107, 851/113, 851/115, 851/116, 851/123, 851/135, 851/136.	40

Fig 1: Diagrammatically showing the various stages involved in the categorization of tea chlorophyll through gamma radiation.

Conclusion
Tea breeding is a continuous process and each trait of interest has their own importance with the breeding objectives. Here, we have categorized 134 diverse tea germplasm on the basis of Chl-a, Chl-b and Carotenoids pigment in terms of high, medium and low. We hope that these categorically diverse populations could participate in the future breeding strategies for the improvement of tea planting materials.

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