

Identification of Anthocyanin Rich Purple Tea in F₁ Progeny Derived through Controlled Hybridization

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ABSTRACT

Anthocyanin rich foods are becoming more popular all over the world. Traditionally tea is manufactured as black tea and green tea in India. Recently, Kenya and Sri Lanka have attempted to produce anthocyanin rich purple tea from the tea plant *Camellia sinensis*. Therefore, through controlled hybridization, F₁ progenies were developed involving an anthocyanin rich clone as one of the female parent for the first time in south India. A total of 51 F₁ progenies were established in the field and screened for the pigments anthocyanin, carotenoids and chlorophyll as well as caffeine. A total of 11 F₁ progenies were identified with high anthocyanin content and the caffeine content is low in these progenies. These identified F₁ progenies are being multiplied through vegetative propagation for commercial production of purple tea with low caffeine content.

Keywords : Controlled hybridization, Clonal selection, Tea germplasm, Plant pigments, Caffeine, Anthocyanin

ANTHOCYANINS are responsible for the blue, purple, red and orange colours in many fruits and vegetables. It has been reported that major sources of anthocyanins are blue berries, cherries, raspberries, strawberries, purple grapes, coloured guava and red wine (Miguel, 2011 and Gowtham Gowda & Shivashankara, 2024). Anthocyanins rich food is becoming more popular amongst the younger generation all over the world. Many researchers have reported that consumption of anthocyanin lower the risk of cardio vascular diseases, diabetics, arthritis and cancer due to their antioxidant, anti-inflammatory properties (Rechner & Kroner, 2005; Wang & Stoner, 2008 and Vidyavathi *et al.*, 2022).

Tea, the most popular beverage is manufactured as black tea which is fully oxidized, oolong tea when partially oxidized and green tea which is non oxidized. In addition to these three types of teas, purple tea

which is also non oxidized is produced from the cultivars derived from the same tea plant (*Camellia sinensis*). Tea cultivars rich in anthocyanin content in the young buds and leaves are used for the production of purple tea. The Tea Research Institutes of Kenya (Kerio *et al.*, 2011) and Sri Lanka (Hopfstock *et al.*, 2024) have already released a few tea clones rich in anthocyanin content for the production of purple tea.

In an earlier study, tea germplasm available in north India were investigated for their suitability for the production of purple tea (Patel *et al.*, 2022). In the same study, the authors have correlated chlorophyll, carotenoids, anthocyanin and caffeine contents to the purple tea quality. UPASI Tea Research Foundation, Tea Research Institute has released 33 tea clones for commercial cultivation in south India. The tea germplasm maintained at UPASI Tea Research Institute is recognized as the 41st National Active

Germplasm Site (NAGS) by NBPGR, New Delhi. The tea clone TRI-2043 developed by Tea Research Institute of Sri Lanka was introduced into south India during the 1960s. The special character of the clone is its high anthocyanin content in the bud and young leaves. Though the clone TRI-2043 was introduced to south India more than six decades back, it was not used in the hybridization programme with the tea clones released by UPASI for developing anthocyanin rich tea cultivars through clonal selection in the F_1 population. Therefore, a controlled hybridization programme was initiated during the flowering season of tea in the year 2017 involving the anthocyanin rich tea clone TRI-2043 as the female parent and UPASI-9, UPASI-28, TRF-1 and Cr-6017 as the male parents. F_1 progenies developed through various parental combinations involving TRI-2043 as the female parent were screened for their anthocyanin, carotenoids, chlorophyll and caffeine contents and reported in the present study.

MATERIAL AND METHODS

Controlled hybridization programme was initiated in the breeding plot established in the UPASI Tea Research Institute Experimental Farm, Valparai, Coimbatore district, Tamil Nadu. The Experimental Farm is located between $10^{\circ} 15.960^{\circ}$ N latitude, $076^{\circ} 58.033$ E longitude, at an altitude of 1050m above mean sea level in the western ghats of South India. The experimental farm receives both the south west and north east monsoons with an average rainfall of 445 cm per year.

Controlled hybridization work was carried out in the early morning hours from December to March during the year 2017. Free grown trees of TRI-2043 were considered as the female parent and free grown trees of UPASI-9, UPASI-28, TRF-1 and Cr-6017 were considered as the male parent (pollen donor). Unopened flowers of UPASI-9, UPASI-28, TRF-1 and Cr-6017 at the balloon stage were collected and the pollen grains were dusted on the stigma of the unopened flowers of the female parent TRI-2043. After dusting the pollens, the flowers were safely bagged and labelled. During the same year in the

month of September / October fully matured seeds were collected and germinated in the tea nursery following the standard nursery practices outlined by Satyanarayana and Sharma (1981).

One year after the nursery life span, all the F_1 progenies were transplanted to the UPASI Experimental Farm in the month of June 2018 coinciding with the onset of southwest monsoon. A total of 51 numbers of F_1 progenies were planted in the UPASI Experimental Farm as per the procedure outlined by Satyanarayana *et al.* (1987).

Screening procedure of F_1 progenies developed through a cross between 1) TRI-2043 (Female parent) x UPASI-9 (Male parent), 2) TRI-2043 (Female parent) x UPASI-28 (Male parent), 3) TRI-2043 (Female parent) x TRF-1 (Male parent) and 4) TRI-2043 (Female parent) x Cr-6017 (Male parent) was carried out for contents of pigments like anthocyanin, carotenoids, chlorophyll and caffeine. Along with the F_1 populations, all the five parental tea clones TRI-2043, UPASI-9, UPASI-28, TRF-1 and Cr-6017 were also vegetatively propagated in the nursery along with the germinating F_1 population and planted in the field for comparison with the F_1 population. Based on the observations, the F_1 population was categorized into progenies with high, intermediate and low in contents of anthocyanin, carotenoids, chlorophyll and caffeine. All the observations were also carried out in all the five parents for comparison.

During the two years of initial establishment of the F_1 progenies in the field, observation on the success rate of F_1 progenies was recorded. A total of 16 progenies were could be established in the combination TRI-2043 x UPASI-9. Ten progenies each in the combinations TRI-2043 x UPASI-28 and TRI-2043 x TRF-1. In the combination TRI-2043 x Cr-6017 15 progenies survived in the field. All the parental combinations were evaluated under Randomized Complete Block Design (RCBD). The surviving F_1 progenies were estimated for contents of anthocyanin, carotenoids, chlorophyll and caffeine from 2020 to 2024 and the mean values were considered for statistical analysis.

Crop shoots comprising three young leaves and the terminal bud were collected from the 51 F_1 progenies developed through various parental combinations as well as the parents maintained in the field germplasm site located in UPASI Tea Research Institute, Valparai. Determination of anthocyanin content was estimated as per the procedure described by Mancinelli and Schwartz (1984). Quantification of carotenoids and chlorophyll content was estimated as per the procedure described by Harbone (1973). Quantification of caffeine content was estimated as per the procedure described by Ronald and Ronald (1991).

Statistical Analysis

Statistical analyses were conducted using IBM SPSS statistics version 23. Data were expressed as the mean values of duplicated tests \pm standard deviation of the mean. A one way ANOVA was used to evaluate the significance of results. For variables for which significant differences ($p \leq 0.05$) were found, Fisher's least significant difference (LSD) was used for comparison of means.

RESULTS AND DISCUSSION

Success Rate of Controlled Hybridization

Success rate of controlled hybridization varied between different parental combinations. Among the four parental combinations, success rate of controlled hybridization was the highest up to 68.18 per cent and 66.33 per cent in the combinations of TRI-2043 x UPASI-9 and TRI-2043 x TRF-1, respectively indicating high compatibility between the parents. Among the other two combinations, a success rate of 42.43 per cent was observed in the combination TRI-2043 x Cr-6017. The fourth parental combination of TRI-2043 x UPASI-28 resulted in the lowest success rate of 39.77 per cent only (Table 1). Such a wide range of difference in the success rate of controlled hybridization in different parental combinations was reported by many tea breeders in the breeding programme to develop tea cultivars with high yield and superior quality (Satyanarayana *et al.*, 1981; Sharma *et al.*, 1981 and Singh, 1982).

Success Rate of Germination in the Tea Nursery

Success rate of germination in the nursery was 68.57 per cent in the combination of TRI-2043 x UPASI-28. Whereas, in all the other three parental combinations, success rate of the germination of seeds was almost the same ranging from 40.00 to 43.67 per cent only (Table 1). Such a narrow difference in the percentage of seed germination could be attributed to the same season and same standard operating procedures followed in the tea nursery for germination of tea seeds.

Success Rate of F_1 Progenies in the Field

Among the F_1 progenies transplanted to the field, the slightly high success rate of F_1 progenies in the field in the combination of TRI-2043 x UPASI-9 could be due to the drought tolerant characters of the male parental clone UPASI-9. A success rate of 53.33 per cent was observed in the combination TRI-2043 x UPASI-9 (Table 1). In the other three combinations of TRI-2043 x TRF-1, TRI-2043 x Cr-6017 and TRI-2043 x UPASI-28, the survival percentage is 37.03 to 41.66 per cent which is almost the same (Table 1).

Differences in Pigments among the F_1 Progenies

The surviving 51 F_1 progenies were observed for the pigments like anthocyanin, carotenoids, chlorophyll and caffeine. Significant differences were observed in anthocyanin, carotenoids, chlorophyll and caffeine among the F_1 progenies (Table 2). Anthocyanin content ranged from 0.018 to 0.256, carotenoids minimum 0.18 to maximum 0.33, chlorophyll A minimum 0.255 to maximum 0.922, chlorophyll B minimum 0.299 to maximum 0.892, total chlorophyll minimum 0.565 to maximum 1.814 and caffeine content minimum 1.76 to maximum 3.34 (Table 2).

Correlation Coefficient among Parameters

Correlation analysis indicated that anthocyanin content was negatively correlated with carotenoids, chlorophyll pigments and caffeine ($P < 0.01$). Significantly positive correlations were also observed between carotenoids with chlorophyll A, chlorophyll B, total chlorophyll and caffeine content ($P < 0.05$) (Table 3). Chlorophyll A, chlorophyll B, total

TABLE 1
Details of controlled hybridization

Parental combination (Female x Male)	Number of flowers pollinated	Number of seeds collected	% of success rate	Number of seeds sown in the nursery	Number of seeds germinated in the nursery	% of success rate in the nursery	Number of progenies transplanted in the field	Number of progenies survived in the field	% of success rate in the field
TRI-2043 X UPASI-9	110	75	68.18	75	30	40.00	30	16	53.33
TRI-2043 X UPASI-28	88	35	39.77	35	24	68.57	24	10	41.66
TRI-2043 X TRF-1	101	67	66.33	67	27	40.29	27	10	37.03
TRI-2043 X Cr-6017	205	87	42.43	87	38	43.67	38	15	39.47
Total	504	264	-	264	119	-	119	51	-

TABLE 2
Variance of pigments and caffeine for all the four parental combinations

Parameters	Source of variance	SS	DF	MS	F value	Mean \pm SD	CV %	Range
Anthocyanin	Among the F ₁ progenies	2.122	50	0.021	25.184	0.093 \pm 0.084	14.61	0.018 – 0.256
	Error	0.344	204					
	Total	2.466	254	-	-	-	-	-
Carotenoids	Among the F ₁ progenies	.308	50	0.004	10.674	0.248 \pm 0.034	13.84	0.18 – 0.33
	Error	0.118	204					
	Total	0.426	254	-	-	-	-	-
Chlorophyll A	Among the F ₁ progenies	9.126	50	0.110	247.234	0.569 \pm 0.189	12.90	0.255 – 0.922
	Error	0.151	204					
	Total	9.276	254	-	-	-	-	-
Chlorophyll B	Among the F ₁ progenies	2.197	50	0.028	39.777	0.559 \pm 0.095	15.10	0.299 – 0.892
	Error	0.225	204					
	Total	2.422	254	-	-	-	-	-
Total chlorophyll	Among the F ₁ progenies	16.996	50	0.207	195.368	1.128 \pm 0.260	11.40	0.565 – 1.814
	Error	.355	204					
	Total	17.351	254	-	-	-	-	-
Caffeine	Among the F ₁ progenies	50.438	50	0.021	81.689	2.56 \pm 0.44	14.32	1.76 – 3.41
	Error	2.519	204					
	Total	52.957	254	-	-	-	-	-

chlorophyll and caffeine content was positively correlated with carotenoids ($P < 0.01$) (Table 3).

Clustering Analysis Based on Anthocyanin Content

Cluster analysis indicated that the 51 F₁ progenies, were classified into three groups based on anthocyanin

content. Group I was characterized with high anthocyanin content and consisted of 11 F₁ progenies. Group II contained 24 F₁ progenies with intermediate and Group III was with lowest anthocyanin content, which contained 16 F₁ progenies (Table 4). However, content of anthocyanin in all these 11 numbers of F₁

TABLE 3
Correlation coefficients of pigments and caffeine content in all the four parental combinations

Parameters	Total anthocyanin	Total carotenoids	Chlorophyll A	Chlorophyll B	Total chlorophyll	Total caffeine
Total anthocyanin	1 -	0.188 **	-0.148 *	-0.102	-0.146 *	-0.239 **
Total carotenoids	-0.188 **	1	0.396 **	0.241 **	0.380 **	0.459 **
Chlorophyll A	-0.148 *	0.396 **	1	0.596 **	0.954 **	0.538 **
Chlorophyll B	-0.102	0.241 **	0.596 **	1	0.810 **	0.341 **
Total chlorophyll	-0.146 *	0.380 **	0.954 **	0.810 **	1	0.520 **
Total caffeine	-0.239 **	0.459 **	0.538 **	0.341 **	0.520 **	1

**Significant at P <0.01 * Significant at P <0.05

progenies was lower than the female parent TRI-2043, but higher than the four male parents UPASI-9, UPASI-28, TFR-1 and Cr-6017. All the 11 F₁ progenies with high anthocyanin content also had lower contents of carotenoids, chlorophyll and also caffeine. When the anthocyanin pigment was the dominating trait, caffeine, carotenoids and chlorophyll content low (Patel *et al.*, 2022). These progenies can be cultivated through vegetative propagation for commercial production of anthocyanin rich purple tea with low caffeine.

Clustering Analysis Based on Carotenoids

All the 51 F₁ progenies were classified into three groups based on their carotenoids content. Group I had a high carotenoid, which included 19 F₁ progenies. Group II had an intermediate carotenoid content, which contained 21 F₁ progenies and Group III (11 F₁ progenies) had the lowest carotenoid content (Table 4). Carotenoids content in all the 19 numbers of F₁ progenies was higher than the five parents TRI-2043, UPASI-9, UPASI-28, TRF-1 and Cr-6017. When the carotenoid pigment was more chlorophyll pigment was also high but anthocyanin pigment was low and caffeine content was intermediate (Patel *et al.*, 2022).

Clustering Analysis Based on Chlorophyll A

On the basis of chlorophyll A, the 51 F₁ progenies, were classified into three groups. Group I was high in chlorophyll A, which contained 19 F₁ progenies. Group II was intermediate in chlorophyll A, which

contained 21 F₁ progenies and Group III was low in chlorophyll A, which contained 11 F₁ progenies (Table 4).

Clustering Analysis Based on Chlorophyll B

According to chlorophyll B, three groups were categorized, consisting of 19, 21 and 11 numbers of F₁ progenies, respectively. Group I had the highest chlorophyll B. Group II contained an intermediate and Group III was categorized with low chlorophyll B content (Table 4).

Clustering Analysis Based on Total Chlorophyll

Based on the total chlorophyll content, the 51 F₁ progenies, were classified into three groups. Group I, which was characterized by a high total chlorophyll content, consisted of 19 F₁ progenies. Group II, which had an intermediate total chlorophyll content, consisted of 21 F₁ progenies and Group III, which had a low total chlorophyll content, consisted of 11 F₁ progenies (Table 4). Chlorophyll content in all the 19 numbers of F₁ progenies is higher when compared to the female parent TRI-2043. When compared to the other four male parents chlorophyll content in the F₁ progenies was almost the same. When the chlorophyll pigment was maximum, anthocyanin was low, carotenoids was high but, caffeine was intermediate. It has been reported that caffeine can alter the content of photosynthetic pigments in plants. Caffeine increase the content of chlorophyll A and chlorophyll B in plants (Hyong *et al.*, 2012).

TABLE 4
Clustering analysis based on various pigments and caffeine content in the F₁ progenies

Parameters	Group	Progeny number	No.of. Progenies	Frequency (%)	mean ± SD	Range
Total Anthocyanin	I	1,4,5,8,20,24,27,29,34,38,46	11	21.57	0.204 ± 0.046a	0.110 - 0.256
	II	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,41,42,43,44,45,49,51	24	47.06	0.069 ± 0.012b	0.052 - 0.091
	III	3,11,12,16,17,18,23,25,31,33,37,39,40,47,48,50	16	31.37	0.037 ± 0.010c	0.018 - 0.049
Total Carotenoids	I	3,11,12,16,17,18,23,25,31,33,37,39,40,41,47,48,49,50,51	19	37.25	0.32 ± 0.01 a	0.31 - 0.33
	II	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,42,43,44,45	21	41.17	0.26 ± 0.02 b	0.21 - 0.30
	III	1,4,5,8,20,24,27,29,34,38,46	11	21.58	0.19 ± 0.01 c	0.18 - 0.20
Chlorophyll A	I	3,11,12,16,17,18,23,25,31,33,37,39,40,41,47,48,49,50,51	19	37.25	0.78 ± 0.06 a	0.706 - 0.922
	II	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,42,43,44,45	21	41.17	0.62 ± 0.06 b	0.503 - 0.699
	III	1,4,5,8,20,24,27,29,34,38,46	11	21.58	0.37 ± 0.07 c	0.255 - 0.490
Chlorophyll B	I	3,11,12,16,17,18,23,25,31,33,37,39,40,41,47,48,49,50,51	19	37.25	0.80 ± 0.06 a	0.710 - 0.892
	II	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,42,43,44,45	21	41.17	0.60 ± 0.04 b	0.527 - 0.660
	III	1,4,5,8,20,24,27,29,34,38,46	11	21.58	0.39 ± 0.05 c	0.299 - 0.410
Total chlorophyll	I	3,11,12,16,17,18,23,25,31,33,37,39,40,41,47,48,49,50,51	19	37.25	1.59 ± 0.10 a	1.455 - 1.814
	II	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,42,43,44,45	21	41.17	1.22±0.05 b	1.162 - 1.306
	III	1,4,5,8,20,24,27,29,34,38,46	11	21.58	0.76 ± 0.09 c	0.565 - 0.899
Total caffeine	I	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,42,43,44	20	39.22	3.21 ± 0.12 a	3.05 - 3.41
	II	3,11,12,16,17,18,23,25,31,33,37,39,40,41,45,47,48,49,50	19	37.25	2.56 ± 0.26 b	2.11 - 2.95
	III	1,4,5,8,20,24,27,29,34,38,46,51	12	23.53	1.93 ± 0.19 c	1.76 - 1.99

Clustering Analysis Based on Caffeine Content

Cluster analysis indicated that the 51 F₁ progenies, were classified into three groups based on caffeine content. Group I was characterized with high caffeine content and consisted of 20 F₁ progenies. Group II

contained 19 F₁ progenies with intermediate and Group III was with low caffeine content, which contained 12 F₁ progenies (Table 4). Caffeine content in all the 20 F₁ progenies was higher than the five parents TRI-2043, UPASI-9, UPASI-28, TRF-1 and Cr-6017. When caffeine was found more both the

TABLE 5
F₁ Progenies identified for high, intermediate and low anthocyanin, carotenoids, total chlorophyll and total caffeine content

Parameters	Characters	Total number of F ₁ progenies	Progeny number	Frequency (%)
Total Anthocyanin	High anthocyanin content	11	1,4,5,8,20,24,27,29,34,38,46	21.56
	Intermediate	24	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,41,42,43,44,45,49,51	47.1
	Low anthocyanin content	16	3,11,12,16,17,18,23,25,31,33,37,39,40,47,48,50	31.34
	Total	51	-	100
Total carotenoids and chlorophyll	High carotenoids and chlorophyll content	19	3,11,12,16,17,18,23,25,31,33,37,39,40,41,47,48,49,50,51	37.25
	Intermediate	21	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,42,43,44,45	41.17
	Low carotenoids and chlorophyll content	11	1,4,5,8,20,24,27,29,34,38,46	21.58
	Total	51	-	100
Total Caffeine	High caffeine	20	2,6,7,9,10,13,14,15,19,21,22,26,28,30,32,35,36,42,43,44	39.22
	Intermediate	19	3,11,12,16,17,18,23,25,31,33,37,39,40,41,45,47,48,49,50	37.25
	Low caffeine	12	1,4,5,8,20,24,27,29,34,38,46,51	23.53
	Total	51	-	100

pigments anthocyanin and carotenoids and chlorophyll were in the intermediate level.

A total of 11 F₁ progenies (21.56 %) were identified for high anthocyanin content followed by 24 (47.10%) for intermediate level of anthocyanin content and 16 (31.34 %) as low anthocyanin content (Table 5).

A total of 19 F₁ progenies (37.25 %) were identified for high carotenoids and chlorophyll content followed by 21 (41.17 %) for intermediate level of carotenoids and chlorophyll content and 11 (21.58 %) as low carotenoids and chlorophyll content (Table 5).

A total of 20 F₁ progenies (39.22 %) were identified for high caffeine content followed by 19 (37.25 %) for intermediate level of caffeine content and 12 (23.53%) as low caffeine content (Table 5).

The present study was carried out to develop F₁ progenies with high anthocyanin content through a controlled hybridization programme involving the tea clone TRI-2043 with high anthocyanin content for the first time as the female parent for the controlled hybridization program. The 11 F₁ progenies with high anthocyanin content identified in this study are being multiplied through vegetative propagation for commercial planting and production of anthocyanin rich purple tea. Since, caffeine content in these 11 numbers of F₁ progenies was also low, these progenies can be cultivated for the production of purple tea with low caffeine content. The study was also useful in developing 20 numbers of F₁ progenies with high caffeine content. These F₁ progenies can be multiplied through vegetative propagation for the commercial cultivation and production of caffeine rich teas.

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