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Unique graft combination of tea, Cr-6017/UPASI-9

S. Balasubramanian*, L. A. Netto and S. Parathiraj

R&D Department, KDHP Co. Pvt Ltd, Munnar 685 612, India

A unique graft combination in tea comprising a quality clone Cr-6017 as a scion and drought hardy high-yielding clone UPASI-9 as root stock was studied under commercial conditions. Average survival rate of cuttings of grafted plants in the nursery was above 85%. Grafted plants of Cr-6017/UPASI-9 were more vigorous and irrespective of the year from planting registered significantly high yields over ungrafted controls. Grafting resulted in increase in the number of branches, branch length and reduction in the branch angle. Grafted plants registered significantly low values of mesomorphy index, vulnerability index, shoot water potential and high values of F_v/F_m. Grafted and ungrafted Cr-6017 plants registered significantly high quantities of total polyphenols and catechins. Variation in the biochemical composition of green leaf and made tea of grafted and control plants of Cr-6017 was statistically non-significant. Quality attributes of teas manufactured from Cr-6017 were superior compared to teas from UPASI-9.

Keywords: Anatomy, biochemical composition, bush architecture, Cr-6017/UPASI-9, drought tolerance, nursery grafting.

GRAFTING has been practised for a long time all around the world. Plantation crops like rubber and horticultural crops like apple, pear, plum, and other woody perennials received most attention in this aspect[1]. In the case of tea, until about 50 years ago, seeds were the only source of propagation. In the absence of suitable mode of vegetative propagation, grafting was tried in the beginning of the last century by certain tea-growing countries to propagate the selected clones primarily for the production of seeds[1–4]. In the early thirties, a simple and economic method of vegetative propagation, in which capacity to root and rapidly form satisfactory nursery plants from single nodal cuttings from the young shoots was demonstrated[5]. Although it took some time to standardize the practice, the method has been widely used since 1950s by most of the tea-growing countries of the world.

Earlier work on grafting in tea mainly aimed at induction of more number of flowers in a shorter time for the production of seeds[1,3,4]. In the case of budding and grafting methods followed in tea, the rootstock had been either established in the field or a seedling from the nursery. During 1971, a unique method was demonstrated for cleft

*For correspondence. (e-mail: balasubramanian.s@kdhptea.co.in)

grafting in tea using fresh cuttings as scion and rootstock in south India for the first time[6]. The main objective of this type of grafting is to develop composite plants comprising the shoot system of a clone with good quality or high yield and the root system of drought-tolerant clones to increase yield per unit area. Today, nursery grafting in tea is being extensively used in south India. However, no data is available on the effect of nursery grafting on a long-term performance of plants under commercial conditions and their response to various factors related to yield and quality. In this context, extensive studies were made on the performance of a promising graft combination of quality tea clone of south India Cr-6017 as a scion and drought hardy high-yielding rootstock UPASI-9 (ref. 7) as a rootstock in large scale commercial planting. The observations made on the performance of the unique graft combination under commercial conditions in comparison with ungrafted rootstock (UPASI-9) and scions (Cr-6017) are discussed in this communication.

All the experiments/observations were carried out in the cultivar proving blocks established during 1992 by the R&D Department of Tata Tea Limited, Munnar (at present Kanan Devan Hills Plantations Company (P) Limited) located above 1400 m msl. Standard operating procedures adopted in south Indian tea plantations[8] were followed in all the experimental plots. Formative pruning was carried out after five years from the date of planting and subsequently, a four-year pruning cycle was adopted.

Composite plants comprising the scions of quality clone Cr-6017 and rootstocks of drought tolerant clone UPASI-9 were established[9]. Mother bushes were pruned at 24 inches during February 1991 and various nutrients and treatments were given as per standard operating procedures. During May 1991, about 100–120-days-old shoots with green semi-hard wood stem and 8–10 leaves were collected and brought to the nursery and the cut ends were immersed in water to prevent desiccation. Nodal cuttings of 4–5 cm length, 3–4 mm diameter with axillary bud and a healthy leaf were collected and the axis of the single leaf cutting selected as stock was about 2.5–3.0 cm above the axillary bud so as to make the cleft. A cleft of 1.5 cm across the middle was made using a blade. In the case of scions, tapering wedge of 1.5 cm long was made by an even and neat slanting cut and then inserted into the cleft until it was firmly held without exposing the cut. A specially designed grafting clip was used to hold the scion and stock intact (Figure 1).

Composite cuttings and single nodal cuttings (controls) were planted in polythene sleeves filled with suitable rooting medium (red soil and sand 1 : 1 with sandy loam texture, pH 4.5–4.9 and EC < 0.05 dsm^{-1}) and growing medium (black soil, sand and compost 3 : 1 : 1 with clay loam texture, pH 4.5–4.9 and EC < 0.05 dsm^{-1}) incubated under a polythene tent to maintain high relative humidity as per the standard operating procedures[8]. In the case of grafted cuttings, after the formation of good root system (about 90 days after planting), the shoots emerging from the rootstocks were removed to facilitate the growth of axillary buds in the scion. In the case of ungrafted plants, cuttings with a healthy leaf and an axillary bud were used. The cuttings were observed for survival percentage, root weight, shoot weight and root–shoot ratio.

During June 1992, well developed plants with 12–14 months of growth, 10–12 leaves and more than 15 inches height were planted in the field adopting single row hedge planting (about 10,000 plants/ha) in separate cultivar proving blocks. The plants were planted at a distance of 2.5 feet from each other and there was a distance of 4 feet between rows in contour lines. The cultivar blocks were brought up to maturity and various cultural operations were undertaken as per the standard operating procedures[8]. Data related to yield and various agronomic traits were monitored periodically and records maintained.

Bush architecture was studied using the method adopted by Ponmurugan *et al.*[10]. Data on position of trunk, branch angle, branch length and number of branches were recorded. Branches were designated as first order ($n + 1$), second order ($n + 2$) and so on till the fifth order ($n + 5$). Branch length was measured using a metric scale and branch angle was measured using a circular ocular meter. The data thus recorded were interpreted for bush architecture.

Comparative anatomy of the recently matured mother leaves and stem portion just above pruning cuts (22 inches from ground level) was studied[11]. Recently matured mother leaves were collected and individually fixed immediately in freshly prepared FAA and the same were used for anatomical observations. Leaf bits of 5 mm^2 were cut from the midportion of the mother leaf including the midrib, dehydrated using TBA liquid paraffin schedule, transferred in paraffin wax and thin sections of 7–10 μm were taken using a rotary microtome. The sections were embedded on glass slides, de-waxed, stained with 'toludine blue-O', mounted and various anatomical observations were made[12]. Observations on the number of stomata per unit area, their breadth and width and diameter of stomatal aperture were restricted to the middle portion of the leaf lamina. Number of pubescents per unit area was studied using recently expanded buds (first expanded leaves). Based on the anatomical observations made on the stem portion just above the pruning cuts (22″), mesomorphy index (MI – dependency on water) and vulnerability index (VI – drought tolerance) were calculated.

Mother leaves (50 nos) were collected randomly from grafted and ungrafted control blocks separately and their initial weight recorded immediately after harvesting. The leaves were dried in a hot air oven set at 103 ± 2°C till constant weight was reached and leaf moisture and dry matter percentage were calculated[13].

Fresh leaf material (500 mg) was taken and ground using a chilled pestle and mortar with 80% aqueous acetone. The aqueous layer was transferred to a clear test tube. The process was repeated till the residue turned

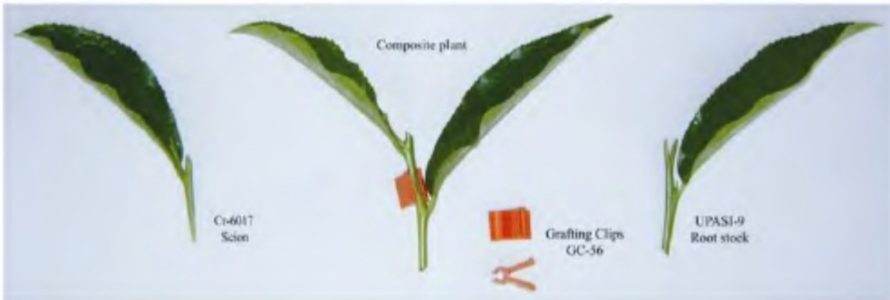

Figure 1. Preparation of composite plants using grafting clips (GC-56).

pure white. The acetone layer with chlorophyll was made up to known volume, and chlorophyll and carotenoids were determined using a UV-Vis spectrophotometer[14].

All the physiological parameters were monitored during drought (January to April). In tea, the mother leaves are a potent source[15], hence all physiological observations were restricted to the leaves. Net photosynthesis rate (P_n), leaf temperature at the time of measurement of P_n, transpiration rate and stomatal conductance were measured using an infrared gas analyser (ADC LCA-3, UK) and an open type Parkinson Leaf Chamber (ADC PLC-3). Water-use efficiency was calculated from the ratio between net photosynthesis rate and rate of transpiration. Shoot water potential was measured using a Dew Point Microvoltmeter (HR 33T, Wescor Inc, Logan Utah) set at dew point mode.

Ex situ sampling method was adopted followed by preconditioning to facilitate more distinct and uniform P–S–M–T transition[16]. Chlorophyll fluorescence was measured using a portable Plant Efficiency Analyser (PEA – Hansatech Instruments Ltd, UK) fitted with a head composed of six light emitting diodes (LED). The dark adapted leaves were exposed to 50% light intensity (1000 μmol m^{-2} s^{-1}) at 690 nm for 1 s and the values were recorded. '0' level fluorescence or the minimal fluorescence yield of a dark adapted leaf (F_0), maximal fluorescence or fluorescence yield (F_m) and the area under the fluorescence curve up to the asymptote (T_{max}) were monitored. From these values, total variable fluorescence ($F_v = F_0/F_m$) and the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) were calculated.

Fresh leaf material (1.0 g) was ground into a paste with 2.5% sulphosalicylic acid and the final volume made up to 25 ml. One millilitre of the filtered solution was mixed with 1.0 ml acid ninhydrin reagent and boiled at 80°C for 60 min. The chromophores thus developed were transferred to toluene and the colour intensity was measured in a UV-Vis spectrophotometer. The values were compared with various concentrations of standard proline[17].

For all the biochemical compositions, freshly plucked shoots consisting of three leaves and a bud were used. Chlorophyll content of the flush shoots (three leaves and a bud) was quantified using 80% acetone as outlined earlier[14].

For the preparation of ethanol extract, 1 g of freshly harvested shoots was ground into a fine paste with warm aqueous ethanol (80%). The extract was allowed to boil on a water bath at 50°C for 10 min. The aqueous ethanol layer was filtered using four folds of muslin cloth. The process was repeated five times and the final volume was made up to 250 ml with 80% ethanol and the same was used for the estimation of amino acids and soluble sugars[17]. Studies on different fractions of catechins and caffeine were carried out using a HPLC fitted with a UV detector and phenyl-hexyl column (Phenomenex Luna, 250 × 4.6 mm, 5 μm) using the method IS 15344–2003 (ref. 18). Technical standards with known purity of various fractions of catechins and caffeine from Sigma Chemicals were used for quantification.

Fresh tea shoots (three leaves and a bud) of grafted plants and ungrafted controls were plucked separately and withered under room temperature for 16 h to reduce the moisture to 60–65%. The withered leaves were passed into a CTC roller four times. The resultant 'dhool' was allowed to ferment (oxidize) at 90% RH in a humidity chamber. Five grams of fermenting 'dhool' was drawn at a time interval of 10 min periodically and the brew was prepared immediately using 100 ml of boiling distilled water. The brew was swirled for 2 min and filtered using cotton plugs. Five millilitres of brew was mixed with 5.0 ml of 2.5% disodium hydrogen phosphate and partitioned with 10 ml of ethyl acetate in a separating funnel. The aqueous ethyl acetate layer was collected and read at 360 nm in a UV-Vis spectrophotometer for theaflavins. The OD obtained at every 10 min for 120 min was fitted into a quadratic curve and the time of influx of OD (maximum TF formation or OFT) was determined[19].

Fresh tea shoots (three leaves and a bud) of grafted and ungrafted plants were plucked separately and withered under room temperature for 16 h to reduce the moisture to 60–65%. The withered leaves were passed into a CTC roller four times and the resultant 'dhool' was allowed to ferment (oxidize) at 90% RH in a humidity chamber for specific optimum fermentation time (the time at which formation of theaflavins reaches the maximum). The fermented 'dhool' was dried in a mini FBD at 105°C for 20 min to final moisture of 3.0%. The resultant tea samples were graded using an automated mechanical sieve and stored in airtight containers and subsequently used for biochemical estimation[20]. The tea samples were also subjected to organoleptic evaluation with the help of professional tea tasters and the scores for various quality attributes were tabulated.

Volatile flavour composition of teas was studied using simultaneous distillation and extraction (SDE) method[21]. Known quantity of tea was boiled in glass distilled water and the volatiles were condensed and partitioned with dichloromethane. The extract thus obtained was concentrated to 1 ml using a rotary evaporator at low vacuum and the volatile flavour compounds studies with gas chromatograph fitted with a flame ionization detector (FID) and a carbowax column (HP Innowax, 30 m length, 0.25 mm ID and 0.25 μm film thickness). From the peak area of individual compound against technical/internal standards, flavour index (FI) was derived.

The analytical data presented here are a result of replicated trials (trial plots – 3 replicates, nursery – 3 replicates with 2000 cuttings and other parameters – minimum 5 replicates each) and the mean values were calculated. Statistical analysis was carried out with SPSS.

In the present study, the most popular tea clone for yield and drought tolerance UPASI-9 released for commercial planting by UPASI Tea Research Institute, Valparai, Tamil Nadu[7] and the popular clone for quality Cr-6017 under south Indian tea growing conditions identified from the Craigmoore Estate, Nilgiris, during 1962 were used. The important characters of the clones are enumerated here.

UPASI-9 is a 'Chinary type' hybrid with vigorous semiorthotropic branches. Leaves are elliptic, large, dark green, venated with wavy margin. Flush shoots are fairly dark, mildly pubescent, slender and moderately easy to harvest. It is a good rooter with uniform growth. It is tolerant to drought, can withstand unfavourable soil reactions and suitable for wide range of climatic conditions. It is the most popular clone of south India in view of its all-round good performance. This clone serves as an excellent rootstock for most of the scions. It is a medium fermenter producing medium quality teas at all elevations. The infusion is slightly dark and open. Liquor will be strong, slightly green and coppery with milk.

Cr-6017 is a 'Cambod type' semi-orthotropic grower with strong frames and more number of lateral branches. Leaves are ovate, dark green and prominently venated in the upper surface. Margin is distinctly serrated with a blunt tip. Flush shoots are medium sized, light green, moderately pubescent and slightly coarse to harvest. Performance in the nursery will be moderate with uneven growth. This clone is moderately tolerant to drought and frost and does well at high and mid-elevation. It is a medium fermenter producing excellent quality teas at all elevations. Teas are greenish black, grainy, dense with coppery/bright infusion and more colour, less green liquor with good body.

Performance of grafted plants and ungrafted controls in the nursery is presented in Table 1. The average survival rate of grafted plants over a period of three years was 87.00% against 83.33% of straight cuttings of Cr-6017 and 90.67% of UPASI-9. The time taken for the plant to attain planting stage was 12–14 months. The grafted plants registered significantly high root weight and total biomass compared to ungrafted controls after 12 months of growth in the nursery.

Yield data recorded from the trial plots of grafted plants as against straight cuttings for 16 consecutive years under large scale commercial planting is presented in Table 2. The yield data recorded during 1993–2009 indicated that the graft combination was superior to plants propagated using straight cuttings of rootstock as well as scion irrespective of the year of planting. The grafted plants registered significantly high yield over both ungrafted Cr-6017 (scions) as well as UPASI-9 (rootstocks). Cumulative yield for 16 years indicated an average crop increase of 28.61% and 8.32% over ungrafted plants of Cr-6017 and UPASI-9 respectively. Average biomass of pruned branches and leaves, and the number of cut ends while pruning (22″ from ground level) during the 17th year from planting (completion of 3rd cycle) are presented in Figure 2. The average biomass per bush and the number of cut ends at 22″ from ground level while pruning were significantly high in the grafted plants when compared to ungrafted Cr-6017 as well as UPASI-9.

Bush architecture of grafted plants in comparison to ungrafted controls is presented in Table 3. The total plucking surface, number of plucking points per unit area and girth of the main stem were significantly high compared to ungrafted Cr-6017. The average/total number of branches of different orders were statistically significant compared to the ungrafted plants of Cr-6017 and UPASI-9; however, the branch length was nonsignificant. In the case of branch angle, the grafted plants registered significantly lower angles compared to ungrafted controls. The observation on bush architecture indicated that grafting resulted in increase in the number of branches, branch length and reduction in the branch angle which ultimately resulted in increase in total plucking surface and number of plucking points per unit area.

Important anatomical parameters of grafted and ungrafted control plants are presented in Table 4. In the case of number of stomata per unit area and their size, difference was nonsignificant. In the case of size of stomatal aperture, the grafted plant registered significantly lower values compared to ungrafted Cr-6017. The stomata in the case of grafted plants were more or less round whereas in the case of ungrafted plants, it was elliptic.

Table 1. Performance of grafted plants in comparison with ungrafted scion and rootstock (12-month-old plants)

Growth parameters	Cr-6017	Cr-6017/UPASI-9	UPASI-9	SD
Survival percentage (average for 3 years)	83.33	87.00	90.67	3.67
Time taken for maturity (months)	12–15	12–14	12–14	–
Root weight (g)	30.04	43.11	33.99	6.70
Shoot weight (g)	33.55	27.41	27.61	3.49
Total biomass (g)	63.59	70.52	61.60	4.68

Table 2. Comparative yield kg/ha for grafts and ungrafted plants (planted during 1992)

	Immature (yield kg/ha)					Cycle I (yield kg/ha)				
Treatments	YR.2	YR.3	YR.4	YR.5	Cycle average	YR.1	YR.2	YR.3	YR.4	Cycle average
Cr-6017	600	851	1174	1992	929	1105	2682	2731	2327	2211
Cr-6017/UPASI-9	662	1305	1421	2253	1131	1255	2937	5910	3256	3340
UPASI-9	495	1059	1936	2365	1175	1262	2772	3266	2821	2530
SD	84	227	389	191	131	89	129	1702	465	582
	Cycle II (yield kg/ha)					Cycle III (yield kg/ha)				
	YR.1	YR.2	YR.3	YR.4	Cycle average	YR.1	YR.2	YR.3	YR.4	Cycle average
Cr-6017	1773	3157	2244	2112	2322	2404	2970	2028	1878	2320
Cr-6017/UPASI-9	2241	4085	2735	2657	2929	2907	3333	2451	2310	2750
UPASI-9	2196	3842	2786	2706	2883	2933	3190	2416	2406	2736
SD	258	481	299	330	338	298	183	234	281	244

The midrib thickness of grafted plants was significantly higher compared to ungrafted Cr-6017 and UPASI-9. Significant difference could not be established in the case of other anatomical features of mother leaves between grafted and ungrafted plants of Cr-6017. In the case of anatomy of branches at 22″ above ground level (while pruning during 17th year), grafted plants registered significantly high values over ungrafted Cr-6017. The thickness of bark of the ungrafted plants of Cr-6017 was significantly high. The number of vessels per unit area was high in the grafted plants compared to ungrafted Cr-6017 and UPASI-9. The diameter and length of the vessels were high in ungrafted control plants. From the recorded values, MI and VI were arrived at and the grafted plants registered significantly low values of MI and VI, which indicate that their dependency on water will be less and they are less vulnerable to adverse drought conditions.

Physiological parameters in the mother leaves of grafted and ungrafted control plants during drought period are presented in Table 5. Leaf dry matter content, chlorophyll and carotenoids were significantly high in the grafted plants compared to controls. Net photosynthesis rate (P_n) and water-use efficiency during drought period were significantly high compared to ungrafted plants. Variable fluorescence (F_v/F_m), a good indicator of stress tolerance, and presence of proline were also significantly higher compared to ungrafted control plants. In the case of shoot water potential (ψ), the grafted plants registered significantly low values compared to the ungrafted plants, indicating their high level of tolerance to unfavourable drought conditions. In general, the grafted plants remained healthy and green during unfavourable drought conditions (January to April) compared to control pants, which showed symptoms of wilting and dormancy.

Important biochemical constituents, which are responsible for the formation of quality attributes of black tea in the shoots ready for harvest (three leaves and a bud), are presented in Table 6. Dry matter content, total chlorophyll and carotenoids in the shoots of grafted plants were significantly high compared to ungrafted Cr-6017. Grafted and ungrafted Cr-6017 registered significantly high quantities of total polyphenols and catechins, which play a crucial role in the formation of quality attributes during manufacture when compared to the ungrafted plants of UPASI-9. The difference in the total polyphenols of grafted and ungrafted Cr-6017 plants was non-significant. Similarly, the differences in the quantity of caffeine as well as optimum fermentation time of grafted and ungrafted Cr-6017 plants were statistically

Table 3. Bush architecture of grafted plants in comparison with ungrafted scion and rootstock

Parameters	Cr-6017	Cr-6017/UPASI-9	UPASI-9	SD
Plucking surface (cm^2)	6326	9901	9304	1915
Plucking points/sq. ft	113.80	129.80	124.60	8.16
Stem circumference (cm)	31.20	37.12	29.40	4.04
Length of main stem (cm)	7.80	5.04	6.00	1.40
Number of branches				
1st order	6.00	6.20	5.40	0.42
2nd order	2.12	2.68	2.00	0.36
3rd order	2.65	2.80	2.72	0.08
4th order	2.62	2.72	3.04	0.22
5th order	2.44	2.64	2.68	0.13
Average	3.17	3.41	3.17	0.24
Total	15.83	17.04	15.84	1.20
Branch length (cm)				
1st order	14.93	21.12	15.62	3.39
2nd order	15.76	13.79	11.18	2.30
3rd order	13.74	15.92	9.32	3.36
4th order	11.75	10.13	9.39	1.21
5th order	11.16	9.10	7.06	2.05
Average	13.47	14.01	10.51	2.46
Total	67.33	70.06	52.57	12.31
Branch angle (°)				
1st order	61.00	53.72	57.36	3.64
2nd order	56.72	50.28	52.37	3.29
3rd order	46.92	41.60	43.68	2.68
4th order	48.60	43.24	41.32	3.77
5th order	41.68	43.04	42.92	0.75
Average	50.98	46.38	47.53	2.83
Total	254.92	231.88	237.65	14.13

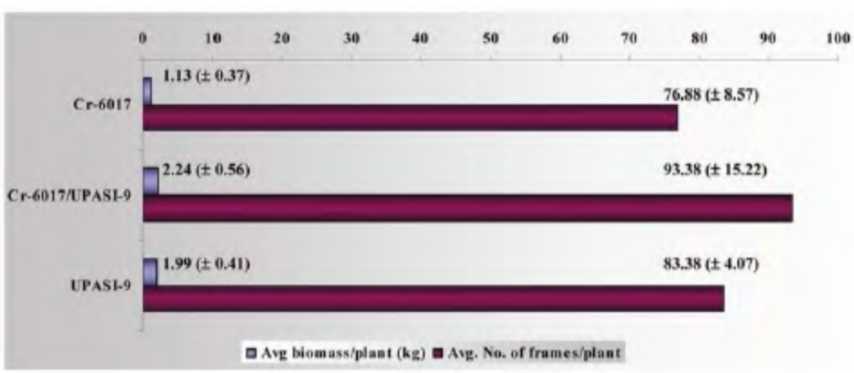

Figure 2. Average biomass/plant (kg) and number of frames (cut ends)/bush recorded while pruning during the completion of 3rd cycle (17th year from planting).

nonsignificant. The observations made on the biochemical composition of shoots clearly indicate that grafting does not influence the quality of scions.

Biochemical composition of CTC black teas manufactured from the shoots harvested from grafted and control plants is presented in Table 7. In most of the biochemical constituents, the difference between grafted and ungrafted plants of Cr-6017 was only marginal and statistically nonsignificant. However, the difference between biochemical constituents of Cr-6017 plants (both grafted and control) was significantly high compared to teas manufactured from UPASI-9 in most of the quality attributes. Teas manufactured from grafted and ungrafted Cr-6017 plants conformed to all regulatory requirements

Table 4. Anatomical similarities of grafted plants in comparison with ungrafted scion and rootstock

Anatomical of mother leaves	Cr-6017	Cr-6017/UPASI-9	UPASI-9	SD
No. of pubescent/mm^2	22.00	22.40	26.80	2.66
No. of stomata/mm^2	148.00	148.40	140.80	4.28
Stomata length (μm)	36.26	35.89	31.45	2.68
Stomata breadth (μm)	25.90	29.97	26.27	2.25
Pore length (μm)	23.31	22.02	21.09	1.12
Pore breadth (μm)	12.95	11.10	14.06	1.50
Midrib thickness (μm)	971.25	1046.25	1020.00	38.06
Vascular bundle breadth (μm)	345.00	420.00	386.25	37.56
Vascular bundle width (μm)	585.00	641.25	660.00	39.03
Total leaf thickness (μm)	330.00	313.13	337.50	12.48
Cuticle thickness (μm)	6.81	7.47	8.29	0.74
Upper epidermis (μm)	18.13	15.91	16.28	1.19
Palisade thickness (μm)	100.64	97.68	100.64	1.71
Palisade width (μm)	15.54	13.32	17.02	1.86
Spongy thickness (μm)	165.76	176.12	187.96	11.11
Spongy width (μm)	22.20	22.20	20.35	1.07
Air space (μm)	22.20	21.46	22.20	0.43
Lower epidermis (μm)	14.06	11.10	11.10	1.71
Anatomy of branches at 22″ from ground level				
Stem diameter (mm)	80.40	90.20	94.13	7.07
Bark thickness (μm)	417.95	404.93	408.48	6.73
Number of vessels/mm	69.67	97.33	76.33	14.44
Vessel length (μm)	336.34	284.59	289.30	28.61
Vessel diameter (μm)	16.28	12.28	13.88	2.01
Mesomorphy index (MI)	0.23	0.13	0.18	0.05
Vulnerability index (VI)	78.60	35.92	52.58	21.51

Table 5. Physiological variations in the mother leaves of grafted plants in comparison with ungrafted scion and rootstock

Physiological features (mother leaf)	Cr-6017	Cr-6017/UPASI-9	UPASI-9	SD
Leaf moisture (%)	72.64	71.52	71.14	0.78
Total chlorophyll (mg/g)	1.17	1.84	1.25	0.37
Chlorophyll *a* (mg/g)	0.94	1.47	0.99	0.29
Chlorophyll *b* (mg/g)	0.23	0.38	0.26	0.08
Chlorophyll *a*/*b*	4.16	3.92	3.84	0.17
Carotenoids (mg/g)	0.30	0.42	0.33	0.06
P_n (μmol min^{-2} m^{-2})	6.80	11.38	8.73	2.30
Transpiration rate (mol s^{-2} m^{-2})	2.65	3.24	4.04	0.70
Water use efficiency	2.57	3.51	2.16	0.69
Stomatal conductance (mol s^{-2} m^{-2})	0.06	0.17	0.18	0.07
Minimal fluorescence yield (F_0)	538.00	502.00	553.00	26.21
Maximal fluorescence yield (F_m)	1501.00	1604.00	1794.00	148.64
Maximum variable fluorescence (F_v)	962.00	1102.00	1241.00	139.50
Variable fluorescence (F_v/F_m)	0.64	0.68	0.69	0.03
Proline (μmol g^{-1})	0.92	1.05	1.10	0.09
Shoot water potential (mvolt bar^{-1})	–8.67	–8.28	–7.32	–0.69
Response to drought	Susceptible	Tolerant	Tolerant	–

like water extract, crude fibre, total ash, water soluble ash, alkalinity of water soluble ash and acid insoluble ash. Grafted and ungrafted Cr-6017 plants registered significantly high value of flavour index (FI) as well as total quantity of volatile flavour compounds when compared to tea manufactured from UPASI-9. These observations indicate that the rootstock does not influence the quality-related attributes of the scion and the scion continues to produce good quality raw material as well as end product in spite of grafting.

CTC black teas manufactured from the grafted and ungrafted controls were subjected for organoleptic evaluation with the help of three individual professional tea tasters and the consolidated results are presented in Table 8. In general, the teas manufactured from the clone Cr-6017 registered better cup characters in terms of score

Table 6. Biochemical composition of flush shoots of grafted plants in comparison with ungrafted scion and rootstock

Biochemical composition	Cr-6017	Cr-6017/UPASI-9	UPASI-9	SD
Dry matter (%)	21.96	22.45	22.05	0.26
Total chlorophyll (mg/g)	0.97	1.16	1.16	0.11
Chlorophyll *a* (mg/g)	0.67	0.83	0.90	0.12
Chlorophyll *b* (mg/g)	0.30	0.33	0.27	0.03
Chlorophyll *a*/*b*	2.20	2.49	3.37	0.61
Carotenoids (mg/g)	0.19	0.23	0.30	0.06
Gallic acid (% by mass)	0.36	0.53	0.44	0.09
(–) Epigallocatechin (% by mass)	7.73	5.88	5.73	1.11
(+) Catechin hydrate (% by mass)	0.13	0.36	0.36	0.14
(–) Epicatechin (% by mass)	1.23	1.16	1.20	0.04
(–) Epigallocatechin gallate (% by mass)	7.60	8.14	7.68	0.29
(–) Epicatechin gallate (% by mass)	3.66	4.39	3.73	0.40
Total polyphenols (% by mass)	20.70	20.46	19.13	0.85
Caffeine (% by mass)	3.64	3.96	3.11	0.43
Soluble sugars (% by mass)	4.31	4.20	4.87	0.36
Free amino acids (% by mass)	0.64	0.77	0.54	0.12
Optimum fermentation time (min)	65.00	65.00	70.00	2.89

Table 7. Biochemical composition of CTC black teas of grafted plants in comparison with ungrafted scion and rootstock

Biochemical parameters	Cr-6017	Cr-6017/UPASI-9	UPASI-9	SD
Total liquor colour (TLC)	3.67	3.79	3.64	0.08
Theaflavins (% by mass)	0.99	1.16	0.89	0.14
Thearubigins (% by mass)	9.02	8.90	9.37	0.24
High polymerized substances (% by mass)	8.66	8.48	9.60	0.60
Caffeine (% by mass)	3.62	3.78	3.72	0.08
TF : TR	9.12	7.67	10.49	1.41
Colour index (CI)	5.60	6.67	4.71	0.99
Briskness index (BI)	21.46	23.49	19.37	2.06
Water extract (% by mass)	40.92	41.00	39.00	1.13
Crude fibre (% by mass)	9.62	9.52	9.05	0.30
Total ash (% by mass)	5.40	5.53	5.12	0.21
Water soluble ash (as% of total ash)	62.04	60.04	58.73	1.67
Alkalinity of water soluble ash (K_2O% as mass)	1.92	1.80	1.76	0.08
Acid insoluble ash (% by mass)	0.07	0.08	0.07	0.01
Flavour index (FI)	3.30	3.96	1.15	1.47

compared to UPASI-9. The infusion of Cr-6017 was bright, which is a good indication for quality. The teas manufactured from grafted plants were marginally superior to ungrafted control plants of Cr-6017; however, the difference was statistically nonsignificant.

It is generally believed that in budded and grafted plants in which two clones are involved with the stock plants unusually older than scion, the economic life of a vegetative propagated plant may be affected by the age of the stock plant and vigour[4]. In nursery grafting of tea, since both rootstock and scion are the same aged cuttings, such an influence by the stock is not likely to occur and the right combination will have a good impact on productivity and quality in the long run[9,22]. In tea plants, the young shoots consisting of the bud and the first two to three leaves are harvested periodically for the manufacture of tea. Hence the commercial productivity of tea is directly related to net assimilation rates and partitioning of assimilates to the shoots rather than the root system is more important than other crops where the partitioning of assimilates between the vegetative organs and the harvested seeds or fruit is important[23]. Bezbaruah and Saharia[24] found that the behaviour of scions was highly influenced by the rootstocks and the grafted scions behaved exactly like the rootstocks during flowering period. In the present study, the grafted plants registered significantly high yield (kg/ha) irrespective of the year from planting and out-yielded ungrafted scions and rootstocks[6]. Moreover, the average biomass of pruned branches and the number of cut ends while pruning (22″ from ground level) during the 17th year (completion of 3rd cycle) were significantly high in the grafted plants compared ungrafted plants of Cr-6017 and UPASI-9.

The influence of rootstock on growth and vigour of the scion, flowering, fruit set, fruit quality, disease resistance and many other characters was studied in a wide range of

Table 8. Taster's evaluation of CTC black teas of grafted plants in comparison with ungrafted scion and rootstock

Quality parameters	Cr-6017	Cr-6017/UPASI-9	UPASI-9	SD
Made tea appearance (1–10)	6.00	5.88	5.13	0.47
Infusion (in words)	Bright	Bright	Fair	–
Infusion (1–10)	8.00	8.00	7.00	0.58
Liquor colour (1–10)	6.00	6.50	5.63	0.44
Liquor brightness (1–10)	6.75	7.00	5.88	0.59
Liquor briskness (1–10)	6.50	6.75	5.88	0.45
Average total score	33.25	34.13	29.50	2.46

crops and the interaction is not as simple as it appears[1]. Studies on rootstock–cultivar interactions on hydraulic conductivity[25] showed that the rootstock controls total growth, whereas the scion controls distribution of growth and the graft was found to influence the movement of substances in the xylem such as ions, water and plant-growth-regulating hormones. This could be attributed to the anatomical changes in the scion especially in the xylem which plays a crucial role in the translocation of substances, particularly minerals and plant growth regulators. The effect of grafting on the anatomical parameters of the leaves was statistically nonsignificant. However, in the case of anatomy of branches above 22″ from the ground level, the grafted plants registered significantly high branch diameter, bark thickness and number of vessels per unit area. In the case of vessel diameter, vessel length, MI and VI, the grafted pants registered significantly low values, which indicate that their dependency on water is less and they are less vulnerable to adverse drought conditions. The observations made by Balasubramanian and Muraleedharan[11] on the anatomy of popular clones of south India indicated that the clones with inherent drought tolerance recorded low values of MI and VI compared to clones which are prone to drought.

Plants adapt themselves to drought situations by reducing transpiration loss through physiological adjustments mainly in the leaves such as regulation of stomata, density of leaf hairs, small leaf area and formation of cutinized layers over the leaf surface[26]. In the present study, observations on various physiological parameters indicated that the grafted plants are physiologically more active compared to ungrafted scion and rootstocks. Shoot water potential (ψ), a measure of the internal water status of a plant and a direct measure of the negative pressure or tension of the sap in the vascular system by which a suction force develops to draw more water[27], was low compared to the ungrafted control plants. The quantity of proline in the grafted plants was significantly high compared to ungrafted plants which will have an important impact on the drought tolerance[28]. On the whole, the grafted plants were physiologically active and showed good level of tolerance to drought against the ungrafted rootstock and scion even under the undesirable environmental conditions during months of severe drought (i.e. January to April).

Studies on grafting of tea clones in Kenya[29,30] indicated that the effect of drought on the grafted plants was significantly low compared to the plants raised by straight cuttings. The drought tolerance index (DTI), which is the comparison between yield recorded during drought years in previous or subsequent drought-free years, was high and positively correlated with drought tolerance. Smith *et al.*[30], Carr[31] and Tuwei *et al.*[32] found that drought-tolerant clones had the least negative ψ values and clones with low values of ψ were tolerant. It was also shown that drought susceptibility of high-yielding clones was associated with the partitioning of a greater proportion of dry matter to the shoot system than that derived from the root system. Drought-tolerant clones are characterized by the partitioning of a greater proportion of dry matter to the root system and frames[22,33].

Classical work[34,35] on chemical basis of quality in black tea indicates that theaflavins (TF) and thearubigins (TR) are two important factors determining the liquoring characteristics of black tea. Both TF and TR are oxidation products of the flavanols and their levels in black tea are usually found to be correlated with colour and strength. The variation in the biochemical constituents of black teas manufactured from grafted and control plants of Cr-6017 was nonsignificant. Studies in water melon[36] indicated that grafting significantly affected plant growth and fruit yield was positively influenced without any effect on quality (fruit index, rind thickness and soluble solids). Studies on grafting in tomato (*Lycopersicon esculentum* Mill.)[37] resulted in more vigorous growth over non-grafted ones and registered more yield in the greenhouse and in the open-field. Quality and qualitative fruit characteristics (pH, Brix percentage, concentration of lycopene or firmness) were not affected by grafting. However, the grafted plants registered higher quantities of calcium, which was attributed to the higher rate of absorption of water and minerals from the soil by roots of the rootstock. The increase in absorption of nutrients can be attributed to the graft plants' tolerance of low temperature and of salinity; enhanced water and inorganic nutrient uptake[38].

The yield of scions increased with the increase in vigour of the stock and the quality attributes (liquor characters) were not affected irrespective of the vigour or quality of the rootstocks. The present study clearly indicates that the unique graft combinations of Cr-6017 on UPASI-9 will be an ideal combination for combining yield and quality, the two underlying factors which determine the profitability and smooth running of the tea industry.

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