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# EVALUATION OF $F_1$ POPULATION DERIVED THROUGH CONTROLLED HYBRIDIZATION FOR DROUGHT TOLERANCE IN TEA

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## ABSTRACT

**Tea-growing regions in South India experience a prolonged drought period from mid-December to mid-April, which significantly impacts the productivity of tea plantations and, in extreme cases, leads to plant mortality. As tea is primarily cultivated under rainfed conditions, developing drought-tolerant cultivars is crucial for sustaining production during dry spells. Despite limited reports of drought tolerance in only three tea clones, this study aims to enhance drought tolerance by creating an $F_1$ population through controlled hybridization. The hybridization involved the introduction of the Japanese tea clone Yabukita into the South Indian tea germplasm. A total of 144 $F_1$ progenies were developed and evaluated for drought tolerance over four years. Key photosynthetic parameters, including stomatal conductance (Gs), water use efficiency (WUE), net photosynthetic rate (Pn), transpiration rate (Tr), and the number of crop shoots produced during the drought season, were assessed. Based on these parameters, 30 drought-tolerant $F_1$ progenies were identified. These findings highlight the potential of hybridization in developing drought-tolerant tea cultivars for regions facing water scarcity.**

**Keywords:** Clonal selection, Controlled hybridization in tea, Photosynthetic parameters, Vegetative propagation

Tea is cultivated across an area of 6,19,773 hectares in the states of Assam, Tripura, and West Bengal in northern India, as well as in Tamil Nadu, Kerala, and Karnataka in southern India. The region experiences significant seasonal variation in rainfall patterns, with droughts being a recurring issue, particularly in southern India. Tea-growing regions in the Western Ghats are notably affected by drought from mid-December to mid-April, a period extending from the cessation of the northeast monsoon. This drought phase typically lasts between 90 to 120 days (Satyanarayana *et al.*, 1992), resulting in substantial crop losses and even the death of tea bushes. One contributing factor to the rising cost of tea production in southern India is the stagnation in yield, which has been attributed to the ageing tea bush population (Ilango *et al.*, 2013). Consequently, replanting the senile, unproductive tea bushes is essential not only for enhancing productivity but also for reducing production costs.

In southern India, approximately 37% of the total annual tea production, which amounts to 236 million kg, is harvested between December and April. This highlights the critical importance of a clonal selection program aimed at developing drought-tolerant tea clones through vegetative propagation. In tea clonal selection, once a single $F_1$ progeny exhibiting a desirable trait is identified, it is propagated in large quantities through vegetative methods and subsequently released for commercial cultivation (Satyanarayana and Sharma, 1991).

The UPASI Tea Research Institute has developed 33 tea clones for commercial cultivation. Among these, only UPASI-2, UPASI-6, and UPASI-9 have been shown to possess drought resistance (Satyanarayana and Sharma, 1991; Satyanarayana *et al.*, 1992). In contrast, the remaining clones were primarily developed for their high yield potential and superior quality (Satyanarayana and Sharma, 1991).

The tea clone *Yabukita*, introduced to southern India in the 1960s, is highly regarded in Japan for its drought tolerance (Matsumoto, 2006). Despite being introduced over six decades ago, *Yabukita* had not been incorporated into hybridization programs aimed at developing drought-tolerant tea cultivars through clonal selection in the $F_1$ population. Consequently, a breeding program was initiated during the tea flowering season of 2016, involving three distinct parental combinations: UPASI-9 × Yabukita, UPASI-2 × Yabukita, and Cr-6017 × Yabukita. Following controlled hybridization, the $F_1$ progenies were evaluated for their drought tolerance over a four-year period in the field.

## MATERIALS AND METHODS

A controlled hybridization program was initiated at the breeding plot established at the UPASI Tea Research Institute Experimental Farm, located in

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Valparai, Coimbatore district, Tamil Nadu. The farm is situated at 10°15.960'N latitude and 76°58.033'E longitude, at an altitude of 1050 meters above mean sea level in the Western Ghats of South India. The soil at the site is sandy loam with a pH of 5.0, and its organic matter content is 3%. Soil moisture, a critical factor in assessing drought stress, directly influences plant water availability and physiological responses. The experimental farm receives both the southwest and northeast monsoons, with an average annual rainfall of 445 cm.

The controlled hybridization work was conducted during the early morning hours from December to March in 2017. Free-grown trees of UPASI-2, UPASI-9, and Cr-6017 were selected as the female parents, while free-grown trees of *Yabukita* were used as the male parent (pollen donor). Unopened flowers of *Yabukita* at the balloon stage were collected, and the pollen grains were applied to the stigma of the unopened flowers of the female parents (UPASI-2, UPASI-9, and Cr-6017). Following pollination, the flowers were carefully bagged and labeled to prevent contamination. In September/October of the same year, fully matured seeds were harvested and subsequently germinated in the tea nursery, following standard nursery practices (Satyanarayana and Sharma, 1981).

One year after their nursery phase, all the $F_1$ progenies were transplanted to the UPASI Experimental Farm in June 2018, coinciding with the onset of the southwest monsoon. The transplanting followed a Randomized Complete Block Design (RCBD), and a total of 144 $F_1$ progenies were planted, in accordance with the procedure outlined by Satyanarayana *et al.* (1987).

The screening procedure for drought tolerance was conducted on the $F_1$ progenies developed from three distinct crosses: 1) UPASI-9 (female parent) × Yabukita (male parent), 2) UPASI-2 (female parent) × Yabukita (male parent), and 3) Cr-6017 (female parent) × Yabukita (male parent). In addition to the $F_1$ populations, all four parental tea clones, UPASI-9, UPASI-2, Cr-6017, and Yabukita, were vegetatively propagated in the nursery alongside the germinating $F_1$ progenies and planted in the field for comparative analysis. Based on the observations, the $F_1$ progenies were categorized into drought-tolerant and drought-susceptible groups. All assessments were also conducted on the parental clones for comparison purposes.

During the initial two years of field establishment, the success rate of the $F_1$ progenies was recorded. The surviving $F_1$ progenies were monitored for key photosynthetic parameters, including stomatal conductance (Gs), water use efficiency (WUE), net photosynthetic rate (Pn), transpiration rate (Tr), and the number of crop shoots per 30 cm². Observations were made during the drought season, from December to April, over a period from 2020 to 2024, and the mean values were used for statistical analysis. The total of 144 $F_1$ progenies was deemed sufficient for the study, providing adequate statistical power to detect significant differences between groups, especially with the use of statistical analysis. As reported by several researchers, the period from December to April constitutes the drought season in southern India (Satyanarayana *et al.*, 1992). Therefore, all photosynthetic parameters were measured during these four months.

## Gas exchanging parameters

Gas exchange parameters were measured on fully matured fourth leaves of the growing crop shoots using a TARGAS-1 PP system. To ensure accurate readings, the leaves were adapted to chamber conditions for a period of 20–30 minutes before five to ten readings were taken per replicate, and the average was calculated. These measurements were conducted between 11:00 AM and 1:00 PM on clear, cloudless days, with the selected leaves being young, fully expanded, healthy, turgid, flat, and uniform in color. Stomatal conductance (Gs), water use efficiency (WUE), net photosynthetic rate (Pn), and transpiration rate (Tr) were recorded during the peak of the drought period, specifically in February and March, when the plants were under severe moisture stress. The number of crop shoots was determined by placing a quadrat of 30 cm² at the center of the canopy of each tea bush during the same months (February and March).

## Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.0. The data were expressed as mean values from duplicate tests, along with the standard deviation (SD) of the mean. Two-way analysis of variance (ANOVA) was employed to evaluate the significance of differences between groups based on two independent variables. This method allows for the analysis of both the interaction between the two variables and the main effects of each variable individually. The significance of the results was assessed at a significance level of $P \leq 0.0001$. Post-hoc analysis using Fisher's Least Significant Difference (LSD) test was performed for cases where significant differences were detected ($P \leq 0.0001$) using two-way ANOVA. The Fisher's LSD test enables pairwise comparisons of the means to identify specific groups that differ significantly from each other. This method is commonly used following ANOVA to control for Type I error when significant differences are found. Standard deviation (SD) was used to represent the variability within each group, indicating the spread of data points around the mean.

## RESULTS AND DISCUSSION

### Success rate of controlled hybridization

The success rate of controlled hybridization varied across different parental combinations. Among the three combinations, the highest success rate of 57.36% was observed in the UPASI-9 × Yabukita pairing (Table 1), indicating a high degree of compatibility between these two parents. In contrast, the UPASI-2 × Yabukita combination yielded a success rate of 30.61%. The third combination, Cr-6017 × Yabukita, exhibited the lowest success rate at 22.6%. The substantial variation in success rates observed across these parental combinations has been documented in previous studies by several tea breeders (Satyanarayana and Sharma, 1981; Sharma *et al.*, 1981; Singh, 1982).

### Success rate of germination in the tea nursery

In all three parental combinations, the seed germination success rate was nearly identical, ranging from 32.69% to 33.34% (Table 1). This narrow variation in germination rates can be attributed to the consistent standard operating procedures applied in the tea nursery for seed germination.

### Success rate of $F_1$ progenies in the field

Among the $F_1$ progenies transplanted to the field, the highest survival rate of 50.77% was observed in the UPASI-9 × Yabukita combination (Table 1). In contrast, the survival rates for the other two combinations, UPASI-2 × Yabukita and Cr-6017 × Yabukita, were similar, ranging from 42% to 43% (Table 1).

### Differences of photosynthetic parameters among the $F_1$ progenies

The 144 surviving $F_1$ progenies were monitored for photosynthetic parameters and the number of crop shoots per 30 cm² during drought conditions over a four-year period. Significant differences were observed in parameters such as net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), water use efficiency (WUE), and the number of crop shoots per 30 cm² among the $F_1$ progenies (Table 2). $F_1$ progenies identified for drought tolerance exhibited higher values for physiological parameters, including stomatal conductance (Gs), water use efficiency (WUE), net photosynthetic rate (Pn), and transpiration rate (Tr) (Table 2). Specifically, stomatal conductance ranged from 25 to 133, water use efficiency varied from 1.15 to 4.62, net photosynthetic rate ranged from 1.24 to 6.39, and transpiration rate ranged from 1.19 to 4.98 (Table 2). Notably, all drought-tolerant $F_1$ progenies were able to sustain growth during periods of moisture stress and produced a higher number of crop shoots per unit area (Table 2).

**Table 1. Success rates of controlled hybridization and progeny survival in the field**

| Parental combination (Female x Male) | No. of flowers pollinated | No. of seeds collected | % success rate | No. of seeds sown in the nursery | No. of seeds germinated in the nursery | % success rate in the nursery | No of $F_1$ progenies transplanted in the field | No. of $F_1$ progenies survived in the field | % success rate in the field |
|---|---|---|---|---|---|---|---|---|---|
| UPASI-9 x Yabukita | 680 | 390 | 57.36 | 390 | 130 | 33.34 | 130 | 66 | 50.77 |
| UPASI-2 x Yabukita | 980 | 300 | 30.61 | 300 | 100 | 33.33 | 100 | 42 | 43 |
| Cr-6017 x Yabukita | 1150 | 260 | 22.6 | 260 | 85 | 32.69 | 85 | 36 | 42 |
| Total | 2810 | 950 | - | 950 | 315 | - | 315 | 144 | - |

**Table 2. Differences of photosynthetic parameters among the $F_1$ progenies**

| Parameters | Source of variance | *Df* | SS | MS | *F Value* | Mean ± SD | CV (%) | Range |
|---|---|---|---|---|---|---|---|---|
| Gs (mmol ($H_2O$ $m^{-2}$ $S^{-1}$) | Among the $F_1$ progenies | 143 | 155338 | 1016 | *56.18* | 78.14 ± 32.03 | 40.99 | 25-133 |
| | Error | 1 | 145327 | - | - | - | - | - |
| | Total | 144 | 310353 | | | | | |
| WUE (A/E) | Among the $F_1$ progenies | 143 | 145.5 | 1.025 | *474.9* | 2.84 ± 1.01 | 35.4 | 1.15-4.62 |
| | Error | 1 | 6.04 | - | - | - | - | - |
| | Total | 144 | 146.6 | | | | | |
| Pn ($CO_2$) $m^{-2}$ $S^{-1}$) | Among the $F_1$ progenies | 143 | 191.8 | 1.70 | *16.71* | 3.20 ± 1.19 | 37.25 | 1.24-6.39 |
| | Error | 1 | 14.65 | - | - | - | - | - |
| | Total | 144 | 243.1 | | | | | |
| Tr (mmol ($H_2O$) $m^{-2}$ $S^{-1}$) | Among the $F_1$ progenies | 143 | 160.7 | 1.139 | *178.30* | 2.97 ±1.05 | 35.55 | 1.19-4.98 |
| | Error | 1 | 37.45 | - | - | - | - | - |
| | Total | 144 | 162.9 | | | | | |
| No.of crop shoots / 30 $cm^2$ | Among the $F_1$ progenies | 143 | 7047 | 49.28 | *156.8* | 19.93±7.08 | 35.51 | 07-38 |
| | Error | 1 | 2134 | - | - | - | - | - |
| | Total | 144 | 7469 | | | | | |

DF: Degrees of freedom, SS: Sum of Square, MS: Mean of Square, SD: Standard Deviation, CV: Coefficient Variation

Drought has emerged as a significant challenge to tea production in South India. Consequently, the objective of the present study was to develop tea clones with enhanced net photosynthetic rate (Pn) and water use efficiency (WUE). Several tea breeders have emphasized that the combination of these two factors is a key goal in high photosynthetic efficiency (HPE) breeding (Fang *et al.*, 2008). In this study, significant variations in photosynthetic parameters were observed among the $F_1$ progenies (Table 2). Three distinct parental combinations were evaluated, resulting in the identification of 30 drought-tolerant $F_1$ progenies within the framework of the HPE breeding program for tea in South India (Tables 4, 5, & 6).

## Correlation coefficient among the photosynthetic parameters and number of crop shoots for the $F_1$ population

Correlation analysis indicated that Pn is positively correlated with WUE and Gs ($P < 0.0001$). Significantly positive correlations were also observed between Tr and Gs, Gs and WUE and WUE and number of crop shoots / 30 $cm^2$ (Table 3).

All four physiological parameters, stomatal conductance (Gs), water use efficiency (WUE), net photosynthetic rate (Pn), and transpiration rate (Tr), were found to be higher in the four parental lines of all three combinations (Table 4). The drought-tolerant characteristics of the four parents (UPASI-9, UPASI-2, Cr-6017, and Yabukita) were further demonstrated by their ability to produce a greater number of crop shoots during the drought period (Table 4).

## Clustering analysis based on Stomatal conductance (Gs) (mmol ($H_2O$) $m^{-2}$ $S^{-1}$)

Hierarchical clustering analysis classified the 144 $F_1$ progenies into six distinct groups based on stomatal conductance (Gs). Group I, characterized by high Gs, comprised 18 $F_1$ progenies. Group II included 25 $F_1$ progenies with relatively high Gs. Group III, with medium Gs, contained 27 F1 progenies. Group IV consisted of 22 $F_1$ progenies with relatively medium Gs. Group V

**Table 3. Correlation coefficients among photosynthetic parameters and number of crop shoots for the $F_1$ population**

| Parameters | Gs | WUE | Pn | Tr | No. of crop shoots / 30 $cm^2$ |
|---|---|---|---|---|---|
| Gs (mmol ($H_2$0) $m^{-2}$ $S^{-1}$) | - | 0.7255**** | 0.6804**** | 0.6811**** | 0.5821**** |
| WUE (A/E) | 0.7255**** | - | 0.6490**** | 0.6620**** | 0.5189**** |
| Pn ($CO_2$) $m^{-2}$ $S^{-1}$) | 0.6804**** | 0.6490**** | - | 0.6032**** | 0.5821**** |
| Tr (mmol ($H_2O$) $m^{-2}$ $S^{-1}$) | 0.6811**** | 0.6620**** | 0.6032**** | - | 0.4872**** |
| No. of crop shoots/ 30 $cm^2$ | 0.5821**** | 0.5189**** | 0.5821**** | 0.4872**** | - |

****Significant at $P < 0.0001$

Table 4. Photosynthetic parameters and number of crop shoots of $F_1$ progenies

| Parents & progenies | GS | WUE | PN | TR | No. of crop shoots |
|---|---|---|---|---|---|
| UPASI-2 | 130 | 4.87 | 5.64 | 4.92 | 29 |
| UPASI-9 | 139 | 4.90 | 5.8 | 4.98 | 30 |
| Cr-6017 | 121 | 4.75 | 5.33 | 4.87 | 32 |
| Yabukita | 137 | 4.98 | 6.81 | 5.18 | 40 |
| PR-1 | 30 | 1.42 | 1.90 | 1.91 | 11 |
| PR-2 | 35 | 1.69 | 2.11 | 1.78 | 10 |
| PR-3 | 126 | 4.00 | 3.51 | 4.54 | 32 |
| PR-4 | 76 | 3.21 | 2.81 | 2.39 | 15 |
| PR-5 | 48 | 1.36 | 1.88 | 1.42 | 10 |
| PR-6 | 96 | 4.21 | 5.31 | 3.28 | 33 |
| PR-7 | 68 | 3.19 | 3.00 | 2.11 | 20 |
| PR-8 | 73 | 3.16 | 2.81 | 2.14 | 19 |
| PR-9 | 103 | 3.37 | 3.84 | 3.85 | 25 |
| PR-10 | 100 | 3.57 | 3.47 | 2.56 | 20 |
| PR-11 | 101 | 3.43 | 3.92 | 3.20 | 24 |
| PR-12 | 57 | 1.34 | 2.15 | 2.29 | 16 |
| PR-13 | 105 | 3.43 | 3.93 | 4.98 | 25 |
| PR-14 | 93 | 3.57 | 3.79 | 3.95 | 27 |
| PR-15 | 100 | 3.36 | 5.47 | 3.76 | 31 |
| PR-16 | 31 | 1.58 | 1.99 | 1.45 | 9 |
| PR-17 | 130 | 4.13 | 3.87 | 3.29 | 23 |
| PR-18 | 121 | 3.24 | 6.00 | 3.72 | 22 |
| PR-19 | 95 | 3.49 | 4.97 | 2.45 | 26 |
| PR-20 | 48 | 2.49 | 2.35 | 2.38 | 19 |
| PR-21 | 51 | 2.35 | 2.51 | 2.16 | 17 |
| PR-22 | 125 | 3.22 | 5.57 | 4.97 | 28 |
| PR-23 | 98 | 3.35 | 4.49 | 4.37 | 25 |
| PR-24 | 110 | 3.58 | 3.91 | 3.20 | 30 |
| PR-25 | 91 | 3.31 | 4.87 | 3.16 | 27 |
| PR-26 | 109 | 2.54 | 3.42 | 3.13 | 25 |
| PR-27 | 49 | 1.54 | 1.81 | 1.35 | 8 |
| PR-28 | 129 | 4.25 | 3.11 | 3.31 | 33 |
| PR-29 | 78 | 4.12 | 3.85 | 2.76 | 32 |
| PR-30 | 68 | 2.39 | 2.77 | 2.82 | 20 |
| PR-31 | 123 | 4.31 | 4.02 | 3.28 | 28 |
| PR-32 | 94 | 4.19 | 4.52 | 3.40 | 23 |
| PR-33 | 133 | 4.62 | 6.39 | 4.98 | 38 |
| PR-34 | 36 | 1.68 | 1.97 | 1.44 | 10 |
| PR-35 | 108 | 3.52 | 4.73 | 3.18 | 26 |
| PR-36 | 53 | 2.39 | 2.28 | 2.35 | 18 |
| PR-37 | 35 | 2.13 | 2.3 | 2.47 | 14 |
| PR-38 | 49 | 2.31 | 2.22 | 2.15 | 19 |
| PR-39 | 128 | 2.61 | 2.53 | 2.84 | 21 |
| PR-40 | 58 | 2.19 | 2.93 | 4.12 | 19 |

| Parents & progenies | GS | WUE | PN | TR | No. of crop shoots |
|---|---|---|---|---|---|
| PR-41 | 34 | 1.44 | 1.97 | 1.62 | 11 |
| PR-42 | 54 | 1.33 | 1.83 | 1.49 | 10 |
| PR-43 | 94 | 3.82 | 3.98 | 3.39 | 24 |
| PR-44 | 127 | 3.4 | 4.52 | 4.10 | 26 |
| PR-45 | 122 | 4.1 | 5.98 | 3.97 | 32 |
| PR-46 | 73 | 1.40 | 1.97 | 1.34 | 13 |
| PR-47 | 34 | 1.57 | 1.86 | 1.41 | 10 |
| PR-48 | 25 | 1.15 | 1.24 | 1.19 | 7 |
| PR-49 | 69 | 2.42 | 2.41 | 4.31 | 18 |
| PR-50 | 32 | 1.59 | 1.85 | 1.54 | 9 |
| PR-51 | 73 | 2.18 | 2.45 | 2.16 | 19 |
| PR-52 | 51 | 2.17 | 5.81 | 2.13 | 31 |
| PR-53 | 89 | 3.58 | 5.21 | 3.11 | 22 |
| PR-54 | 35 | 1.57 | 1.81 | 1.+66 | 9 |
| PR-55 | 91 | 3.3 | 4.21 | 3.4 | 21 |
| PR-56 | 104 | 4.38 | 4.91 | 3.63 | 34 |
| PR-57 | 131 | 3.1 | 4.38 | 4.68 | 22 |
| PR-58 | 97 | 3.09 | 3.98 | 3.37 | 22 |
| PR-59 | 125 | 4.27 | 4.21 | 3.33 | 35 |
| PR-60 | 94 | 3.12 | 4 | 4.21 | 22 |
| PR-61 | 87 | 4.35 | 4.93 | 3.3 | 35 |
| PR-62 | 55 | 2.28 | 5.69 | 2.68 | 19 |
| PR-63 | 95 | 3.1 | 4.15 | 3.16 | 25 |
| PR-64 | 71 | 2.36 | 2.81 | 2.47 | 18 |
| PR-65 | 51 | 2.39 | 2.29 | 2.18 | 19 |
| PR-66 | 102 | 3.20 | 4.27 | 3.21 | 36 |
| PR-67 | 34 | 1.42 | 1.38 | 1.33 | 12 |
| PR-68 | 52 | 2.38 | 2.64 | 4.67 | 19 |
| PR-69 | 95 | 3.11 | 3.97 | 2.98 | 20 |
| PR-70 | 113 | 3.18 | 4.34 | 3.97 | 21 |
| PR-71 | 108 | 3.30 | 3.94 | 3.51 | 26 |
| PR-72 | 94 | 4.05 | 4.31 | 2.81 | 32 |
| PR-73 | 73 | 2.5 | 2.31 | 4.35 | 18 |
| PR-74 | 30 | 1.56 | 1.68 | 1.51 | 12 |
| PR-75 | 118 | 4.03 | 4.21 | 3.15 | 34 |
| PR-76 | 100 | 3.21 | 3.79 | 4.21 | 30 |
| PR-77 | 50 | 2.14 | 1.99 | 1.83 | 17 |
| PR-78 | 95 | 1.37 | 4.04 | 4.00 | 25 |
| PR-79 | 57 | 1.68 | 2.62 | 2.14 | 20 |
| PR-80 | 45 | 1.56 | 1.72 | 1.98 | 13 |
| PR-81 | 68 | 2.47 | 2.78 | 2.55 | 10 |
| PR-82 | 131 | 4.48 | 4.99 | 4.86 | 35 |
| PR-83 | 37 | 2.52 | 2.48 | 2.79 | 15 |
| PR-84 | 49 | 2.42 | 2.97 | 2.32 | 7 |

| Parents & progenies | GS | WUE | PN | TR | No. of crop shoots |
|---|---|---|---|---|---|
| PR-85 | 55 | 4.11 | 2.65 | 2.72 | 34 |
| PR-86 | 26 | 1.21 | 1.32 | 1.21 | 8 |
| PR-87 | 110 | 3.68 | 3.21 | 4.58 | 28 |
| PR-88 | 66 | 2.61 | 2.25 | 2.24 | 9 |
| PR-89 | 129 | 2.48 | 2.74 | 3.12 | 14 |
| PR-90 | 115 | 4.01 | 2.49 | 4.31 | 30 |
| PR-91 | 69 | 2.24 | 2.96 | 3.57 | 19 |
| PR-92 | 127 | 3.14 | 4.25 | 2.85 | 31 |
| PR-93 | 30 | 1.35 | 1.78 | 1.34 | 10 |
| PR-94 | 29 | 1.55 | 1.83 | 1.48 | 9 |
| PR-95 | 125 | 4.28 | 2.77 | 2.59 | 34 |
| PR-96 | 109 | 3.45 | 4.38 | 4.98 | 32 |
| PR-97 | 49 | 2.30 | 2.71 | 1.54 | 17 |
| PR-98 | 52 | 2.19 | 2.21 | 4.37 | 10 |
| PR-99 | 28 | 1.24 | 1.79 | 1.42 | 11 |
| PR-100 | 122 | 3.27 | 3.65 | 3.9 | 29 |
| PR-101 | 87 | 2.64 | 2.34 | 3.32 | 12 |
| PR-102 | 74 | 4.37 | 2.34 | 4.81 | 19 |
| PR-103 | 77 | 1.61 | 1.42 | 1.34 | 10 |
| PR-104 | 97 | 4.23 | 2.46 | 3.38 | 33 |
| PR-105 | 107 | 3.16 | 3.48 | 3.46 | 18 |
| PR-106 | 70 | 1.28 | 1.84 | 1.90 | 11 |
| PR-107 | 94 | 4.00 | 2.43 | 3.95 | 33 |
| PR-108 | 120 | 3.3 | 3.96 | 3.72 | 27 |
| PR-109 | 32 | 4.22 | 2.02 | 4.00 | 32 |
| PR-110 | 106 | 3.38 | 3.92 | 3.17 | 28 |
| PR-111 | 30 | 1.45 | 2.54 | 2.15 | 19 |
| PR-112 | 66 | 2.34 | 1.37 | 2.49 | 29 |
| PR-113 | 45 | 1.48 | 2.28 | 2.76 | 23 |
| PR-114 | 84 | 3.19 | 3.31 | 3.49 | 27 |
| PR-115 | 109 | 3.12 | 4.52 | 3.45 | 22 |
| PR-116 | 51 | 2.10 | 2.42 | 2.31 | 24 |
| PR-117 | 38 | 4.31 | 1.6 | 2.24 | 34 |
| PR-118 | 101 | 3.57 | 4.17 | 4.59 | 20 |
| PR-119 | 44 | 1.99 | 2.97 | 4.71 | 10 |
| PR-120 | 98 | 4.16 | 4.15 | 3.62 | 32 |
| PR-121 | 97 | 3.30 | 4.31 | 4.13 | 34 |
| PR-122 | 57 | 4.00 | 4.18 | 3.11 | 32 |
| PR-123 | 42 | 1.37 | 1.52 | 1.66 | 17 |
| PR-124 | 77 | 2.21 | 2.54 | 2.71 | 22 |
| PR-125 | 29 | 1.69 | 2.54 | 1.49 | 24 |
| PR-126 | 107 | 4.28 | 3.67 | 3.82 | 31 |
| PR-127 | 40 | 1.28 | 2.80 | 2.43 | 10 |
| PR-128 | 33 | 1.51 | 3.20 | 2.12 | 25 |
| PR-129 | 105 | 4.00 | 4.00 | 1.82 | 33 |
| PR-130 | 117 | 3.27 | 3.52 | 3.52 | 10 |
| PR-131 | 28 | 1.15 | 1.19 | 1.20 | 7 |
| PR-132 | 85 | 4.08 | 3.38 | 3.97 | 31 |
| PR-133 | 110 | 3.20 | 3.81 | 4.18 | 20 |
| PR-134 | 47 | 2.17 | 2.68 | 2.58 | 21 |
| PR-135 | 113 | 4.32 | 4.21 | 4.32 | 34 |
| PR-136 | 120 | 4.39 | 4.81 | 4.81 | 32 |
| PR-137 | 64 | 2.10 | 1.74 | 2.34 | 19 |
| PR-138 | 73 | 1.32 | 2.91 | 2.27 | 18 |
| PR-139 | 39 | 3.35 | 4.77 | 2.14 | 19 |
| PR-140 | 89 | 3.21 | 2.62 | 3.61 | 18 |
| PR-141 | 69 | 3.40 | 2.87 | 4.63 | 9 |
| PR-142 | 42 | 2.41 | 1.91 | 1.37 | 21 |
| PR-143 | 94 | 4.31 | 3.15 | 3.44 | 16 |
| PR-144 | 79 | 2.13 | 1.22 | 1.68 | 10 |

PR: Progeny number, Gs: Stomatal conductance (mmol ($H_2O$) $m^{-2}$ $S^{-1}$), WUE: Water use efficiency (A/E), Pn: Net photosynthetic rate (($CO_2$) $m^{-2}$ $S^{-1}$), Tr: Transpiration rate (mmol ($H_2O$) $m^{-2}$ $S^{-1}$), No. of crop shoots: Number of crop shoots/ 30 $cm^2$

was categorized by low Gs, containing 27 $F_1$ progenies, while Group VI included 25 $F_1$ progenies with relatively low Gs (Table 5).

## Clustering analysis based on water use efficiency (WUE) (A/E)

The 144 F1 progenies were classified into four groups based on their water use efficiency (WUE). Group I, characterized by high WUE, included 31 F1 progenies. Group II, with relatively high WUE, contained 42 F1 progenies. Group III, exhibiting medium WUE, comprised 40 F1 progenies. Group IV, with the lowest WUE values, consisted of 31 F1 progenies (Table 5).

## Clustering analysis based on Net Photosynthetic rate (Pn) (($CO_2$) $m^{-2}$ $S^{-1}$)

Based on the photosynthetic rate (Pn), the 144 $F_1$ progenies were classified into four groups. Group I, with high Pn, included 30 $F_1$ progenies. Group II, with relatively high Pn, contained 41 $F_1$ progenies. Group III, exhibiting medium Pn, comprised 39 $F_1$ progenies. Group IV, characterized by the lowest Pn values, consisted of 35 $F_1$ progenies (Table 5).

## Clustering analysis based on Transpiration rate (Tr) (mmol ($H_2O$) $m^{-2}$ $S^{-1}$)

Based on transpiration rate (Tr), the 144 $F_1$ progenies were categorized into four groups, consisting of 22, 46,

**Table 5. Clustering analysis based on photosynthetic parameters and number of crop shoots for the $F_1$ population**

| Parameters | Group | Progeny number | No. of Progenies | Frequency (%) | mean ± SD | Range |
|---|---|---|---|---|---|---|
| GS (mmol ($H_2O$) $m^{-2}$ $S^{-1}$) | I | 3,17,18,22,28,31,33,39,44,45,57,59,82, 89,92,95,100,108 | 18 | 12.51 | 125.22 ± 4.40[a] | 121-133 |
| | II | 9,10,11,13,24,26,35,56,66,70,71,75,87,90, 96,105,110,115,118,126,129,130,133, 135,136 | 25 | 17.36 | 108.08 ± 4.36[b] | 100-120 |
| | III | 6,14,15,19,23,25,32,43,53,55,58,60, 61,63,69,72,76,78,101,104,107, 114,120,121,132,140,143 | 27 | 18.75 | 93.44 ± 4.34[c] | 84-100 |
| | IV | 4,7,8,29,30,46,49,51,64,73,81,88,91,102, 103,106,112,124,137,138,141,144 | 22 | 15.27 | 72.18 ± 4.28[d] | 64-79 |
| | V | 5,12,20,21,27,36,38,40,42,52,62,65,68, 77,79,80,84,85,97,98,113,116,119,122, 123,134,142 | 27 | 18.75 | 50.15 ± 4.23[e] | 42-58 |
| | VI | 1,2,16,34,37,41,47,48,50,54,67, 74,83,86,93,94,99,109,111,117, 125,127,128,131,139 | 25 | 17.36 | 32.40 ± 3.89[f] | 25-39 |
| WUE (A/E) | I | 3,6,15,28,29,33,45,52,56,59,61,66 72,75, 82,85,91,95,96,104,107,109,117,120,122, 126,129,132,135,136,143 | 31 | 21.53 | 4.23 ± 0.18[a] | 4.00-4.62 |
| | II | 4,7,8,9,10,11,13,14,17,18,19,22,23,24,25, 35,43,44,53,55,57,58,60,63,69,70,71, 76, 87,92,100,102,105,108,110,114,115,118, 121,130,133,141 | 42 | 29.17 | 3.31 ± 0.17[b] | 3.09-3.82 |
| | III | 2,12,20,21,26,30,31,32,36,37,38,39,40, 49,51,62,64,65,68,73,77, 81,83,84,88, 89,90,97,98,101,112,116,119,124,134, 137, 139,140,142,144 | 40 | 25.69 | 2.38 ± 0.15[c] | 2.13-2.64 |
| | IV | 1,5,16,27,34,41,42,46,47,48,50,54,67, 74, 78,79,80,86,93,94,99,103,106,111, 113,123,125,127,128,131,138 | 31 | 23.61 | 1.45 ± 0.13[d] | 1.15-1.69 |
| PN (($CO_2$) $m^{-2}$ $S^{-1}$) | I | 3,6,15,28,29,33,45,52,56,59,61,66,72,75, 82,85,91,95,96,104,107,109,117,120,122, 126,129,132,135,136 | 30 | 20.83 | 5.71 ± 0.35[a] | 4.00-6.39 |
| | II | 4,7,8,9,10,11,13,14,17,18,19,22,23,24, 25,35,43,44,53,55,57,58,60,63,69,70,71, 76,87,92,100,102,105,108,110,114,115, 118, 121, 130,133,143 | 41 | 27.79 | 3.64 ± 0.30[b] | 3.00-3.92 |
| | III | 2,12,20,21,26,30,31,32,36,37,38,39,40, 49,51,62,64,65,68,73,77,81,83,84,88,89, 90,97,98,101,112,116,119,124,134,137, 139,140,141 | 39 | 30.55 | 2.55 ± 0.25[c] | 2.11- 2.93 |
| | IV | 1,5,16,27,34,41,42,46,47,48,50,54,67,74, 78,79,80,86,93,94,99,103,106,111, 113, 117,123,125,127,128,131,138,142,144 | 34 | 20.83 | 1.70 ± 0.24[d] | 1.24- 1.99 |
| TR (mmol ($H_2O$) $m^{-2}$ $S^{-1}$) | I | 13,22,23,33,40,44,49,57,60,68,73,76,78, 82,87,90,96,98,102,109,133,135 | 22 | 18.75 | 4.47 ± 0.30[a] | 4.10-4.98 |
| | II | 3,6,9,11,14,15,17,18,24,25,26,28,31,32, 35,43,45,53,55,56,58,59,61,63,66,70,71, 75,89,91,100,101,104,105,107,108,110, 114,118,120,121,126,130,132,136,143 | 46 | 31.95 | 3.47 ± 0.27[b] | 3.11-3.95 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | III | 4,7,8,10,12,19,20,21,29,30,36,37,38,39, 51,52,62,64,65, 69,72,79,81,83,84,85,88, 92,95,111,112,113,116,117,119,122,124, 127,128,134,137,138,139, 140,141 | 45 | 28.47 | 2.44± 0.26[c] | 2.13-2.84 |
| | IV | 1,2,5,16,27,34,41,42,46,47,48,50,54,67, 74,77,80,86,93,94,97,99,103,106,115, 123,125,129,131,142, 144 | 31 | 20.83 | 1.52 ± 0.21[d] | 1.19-1.91 |
| No.of crop shoots/ 30cm$^2$ | I | 3,6,15,28,29,33,45,52,56,59,61,66,72, 75,82,85,95,96,104,107,109,117,120,121, 122,126,129,132,135,136 | 30 | 31.25 | 31.42 ± 4.72[a] | 31-38 |
| | II | 4,7,8,9,10,11,13,14,17,18,19,22,23,24, 25,35,43,44,53,55,57,58,60,63,69, 70,71, 76,87,92,100,102,105,108,110,114,115, 118,133,143 | 40 | 27.78 | 25.97 ± 3.56[b] | 21-30 |
| | III | 2,12,20,21,26,30,31,32,36,37,38,39,40, 49,51,62,64,65,68,73,77,81,83,84,88,89, 90,91,97,98,101,112,116,119,124,130, 134,137,138,139,140, 141 | 42 | 21.53 | 16.75 ± 2.96[c] | 11-20 |
| | IV | 1,5,16,27,34,41,42,46,47,48,50,54,67,74, 78,79,80,86,93,94,99,103,106,111,113, 123,125,127,128,131,142,144 | 32 | 19.44 | 9.12± 1.03[d] | 07-10 |



**Table 6. $F_1$ progenies identified for drought tolerance, intermediate and drought susceptible based on net photosynthetic rate (($CO_2$) $m^{-2}$ $S^{-1}$) (Pn), water use efficiency (A/E) (WUE) and number of crop shoots**

| S. No. | Characters | Total number of $F_1$ progenies | Progeny number | Frequency (%) |
|---|---|---|---|---|
| 1 | Drought tolerant | 30 | 3,6,15,28,29,33,45,52,56,59,61,66,72,75,82,85,91,95,96, 104,107,109,117,120,122,126,129,132,135,136 | 20.84 |
| 2 | Relatively Drought tolerant | 44 | 7,8,9,10,11,13,14,17,18,19,22,23,24,25,34,35,43,44,53, 55,57,58,60,63,69,70,71,76,87,92,100,102,105,108,110, 114,115,118,121,130,133,141,143,144 | 30.55 |
| 3 | Intermediate | 39 | 2,12,20,21,26,30,31,32,36,37,38,39,40,49,51,62,64,65,6 8,73,77,81,83, 84,88,89,90,97,98,101,112,116,119,124, 134,137,139,140,142 | 27.08 |
| 4 | Drought susceptible | 31 | 1,5,16,27,34,41,42,46,47,48,50,54,67,74,78,79,80,86,93, 94,99,103,106, 111,113,123,125,127,128,131,138 | 21.53 |
| Total | | 144 | - | 100 |

45, and 31 progenies, respectively. Group I exhibited the highest transpiration rate, while Group II contained progenies with relatively high transpiration rates. Group III included progenies with medium transpiration rates, and Group IV was characterized by a low transpiration rate (Table 5).

## Clustering analysis based on number of crop shoots / 30 cm²

Based on the number of crop shoots per 30 cm², the 144 $F_1$ progenies were classified into four groups. Group I, characterized by a high number of crop shoots per 30 cm², comprised 30 $F_1$ progenies. Group II, with a relatively high number of crop shoots per 30 cm², included 40 $F_1$ progenies. Group III, containing a medium number of crop shoots per 30 cm², consisted of 42 $F_1$ progenies. Group IV, with a low number of crop shoots per 30 cm², contained 32 $F_1$ progenies (Table 5).

Luo *et al.* (1995) reported a significantly positive correlation between the biomass of tea plants and net photosynthetic rate (Pn). Similarly, it has been established that high water use efficiency (WUE) is a key factor in drought resistance in other crops (Zhu and Xu, 2005). Based on the clustering analysis of stomatal conductance (Gs), WUE, Pn, transpiration rate (Tr), and the number of crop shoots per 30 cm² presented in Table 5, all 144 $F_1$ progenies developed through controlled hybridization involving the three parental combinations were classified as drought tolerant, relatively drought tolerant, intermediate, or drought susceptible (Table 6). For the identification of $F_1$ progenies with drought tolerance, two crucial photosynthetic parameters, net photosynthetic rate (Pn) and water use efficiency (WUE), were used as the basis. A total of 30 $F_1$ progenies (20.84%) were identified as drought tolerant, followed by 44 progenies (30.55%) categorized as relatively drought tolerant, 39 progenies (27.08%) as intermediate, and 31 progenies (21.53%) as drought susceptible (Table 6).

In the present study, for the first time, an effort was made to develop $F_1$ progenies with drought resistance through a controlled hybridization program involving the drought-tolerant clone Yabukita, which was introduced to South India from Japan. A total of 30 $F_1$ progenies were identified as drought tolerant based on two key photosynthetic parameters, net photosynthetic rate (Pn) and water use efficiency (WUE), as well as their ability to produce a higher number of crop shoots during the drought season. This controlled hybridization program also marks the first attempt in South India to develop high photosynthetic efficiency breeding (HPE) in tea. All 30 drought-tolerant $F_1$ progenies have been individually propagated through vegetative methods in large numbers and are being planted in the field separately for further screening of their quality.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

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