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Genotypic variation of Physiological and Biochemical traits in new elite arabica (*Coffea arabica* L.) Coffee hybrids under different soil moisture regimes

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ABSTRACT

A study has been done using eight elite arabica hybrids of coffee for assessing genotypic differences in physiological and biochemical traits. Arabica genotypes exhibit variability in their sensitivity to moisture stress. Under the crop's adaptive mechanism, abiotic challenges may modify not only the overall expression of genetic traits but also the physiological processes of plants. Identification and categorization of drought-resistant plant species would then be made easier by an understanding and critical assessment of physiological changes, including photosynthetic characteristics brought on by soil moisture stress. Therefore, new elite arabica hybrids were studied for their differences in growth and development, biochemical traits and carbon exchange rate (CER) in different soil moisture regimes at Central Coffee Research Institute. During the study S.4813, S.4820 and S.4814 were found to be physiologically efficient genotypes with high Carbon exchange rates types. These genotypes also showed better development and growth in comparison to other genotypes with high leaf areas, leaf dry weight, specific leaf weight (SLW) as well as specific leaf area (SLA), which play a direct role in higher photosynthesis production of a plant. Further, these genotypes were also found to have high antioxidant enzyme activity and other biochemical parameters like high carbohydrate content, higher chlorophyll fractions and epicuticular wax content. The study revealed that S.4814, followed by S.4813 and S.4820, are found to be superior genotypes with higher stress adaptability and with potential to yield more under abiotic stress conditions. Hence, these elite hybrids could be used in further breeding programs to develop drought tolerance genotypes.

Keywords: *Coffee, genotypes, biochemical traits, gas exchange parameters, antioxidants, abiotic stress, climate change, breeding programs*

1. INTRODUCTION

Coffee ranks as the second most significant commodity in international trade, following petroleum products. It is cultivated in over 80 nations within both subtropical and tropical regions, significantly contributing to the national economy of these nations. Coffee is a vital agricultural crop, yielding over 6.5 million tons of green beans annually from over 11 million hectares, contributing over 9 billion USD to international trade. High-quality Arabica coffee constitutes around 60 per cent of global production, whilst Robusta comprises the remaining 40 per cent. Coffee is cultivated in regions of India with substantial annual rainfall of over 2000 mm; nonetheless, it experiences moisture stress for three to six months of the season under South Indian conditions [31]. During periods of extended severe drought, floral atrophy is observed, leading to diminished yields. The detrimental impacts of drought can be mitigated with irrigation; however, in the majority of coffee farms in India, irrigation facilities are absent. Robusta coffee responds to irrigation considerably better than Arabica coffee. The second option is to grow drought-tolerant cultivars with higher rates of carbon absorption and water use efficiency. Numerous economically significant arabica cultivars exhibit considerable susceptibility to drought [2; 30]. The development of cultivars exhibiting high vigor and drought tolerance is a protracted endeavor, with limited advancements observed in India and globally.

Additionally, arabica genotypes exhibit variability in their sensitivity to moisture stress [32; 8] . In addition to the overall expression of the genetic characteristics the physiological processes of plants may be modified by abiotic stresses under the adaptive mechanism of the crop. Comprehension and critical evaluation of physiological alterations, including photosynthetic traits resulting from soil moisture stress, would subsequently facilitate the identification and classification of drought-resistant and susceptible plant kinds. Based on studies on osmoregulation, coffee genotypes have been categorized as drought-resistant and susceptible cultivars [31; 33]. Breeders all over the world are making efforts to increase diversity in arabica by tapping the germplasm available, making different crosses and generating new hybrids by various breeding and physiological approaches. At Central Coffee Research Institute (CCRI), India, the Division of Plant Breeding and Genetics, developed new elite hybrids by breeding approach and planted in the field.

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The present study aims to evaluate these elite hybrids for physiological and biochemical parameters under different moisture regimes and to determine whether these plants are capable of withstanding abiotic stress.

2. MATERIALS AND METHODS

2.1 Site selection

The investigation was conducted during 2021-22 and 2022-23 at Chikkamagaluru's Central Coffee Research Institute (CCRI), India, to evaluate the variations of physiological and biochemical traits in new arabica coffee hybrids under different soil moisture regimes. The experiment consisted of eight new promising arabica hybrids viz., S.4808, S.4202, S.4813, S.4820, S.5817, S.4814 and S.4904 are compared with station-released popular cultivar Sln.9. The average maximum temperature observed during the experimental period was 28.6°C, while the average lowest temperature was 17.11°C. The total hours of sunshine ranged from 2.5 to 8.4 hours per day. The soil's water retention capacity at 100% field capacity was 21%. The experimental design used was a CRBD (complete randomized block design) with 3 replications.

2.2 Plant and Soil sample collection

From each treatment, five plants of the same size were chosen at random and tagged to capture different growth parameter observations at different phases. Using the gravimetric approach, the soil moisture content under stress conditions was ascertained. The gravimetric method of soil moisture content determination is most commonly used in which a known weight of wet soil is oven-dried at 100-110ºC for 48 hours, and the moisture removed is calculated. Soil moisture content is expressed as wet weight percentage or volume percentage. The term soil moisture deficit refers to the amount of rain needed to bring the soil moisture content back to field capacity. The maximum quantity of water that a potted plant may be watered without leaking is known as field capacity (SWD=0), this is the volume of water the soil can retain in defiance of gravity [24].

SWD = [{Moisture content at Field Capacity - Present Moisture content} × Bulk density × depth of soil]/100

2.3 Gas exchange parameters

A moveable photosynthesis and fluorescence system (LI-COR, LI-6400 USA) was used to measure the gas exchange parameters. The characteristics of the leaf chamber were kept constant during the trial. The boundary layer resistance in the leaf chamber was 0.08 $m^2 s\ mol^{-1}$, and the gas flow rate was set at 250ml min^{-1}. The atmospheric CO_2 concentration was between 330 and 340 vpm when the CO_2 mode was set to ambient. Before the measurements, the instrument's CO_2 and H_2O calibrations were made.

2.4 Biophysical parameters

Ten leaves (4^{th} pair, fully matured) were plucked from the selected plants. Each leaf's length and width were measured, and the Arabica correction factor (0.63) was applied [7]. The term specific leaf area(SLA), which is measured in dm^2g^{-1}, refers to the average growth in leaf area per unit of leaf dry weight. [16] formula was used to achieve the calculation. The average leaf weight per unit area, measured in grams per square meter, is known as specific leaf weight (SLW) (leaf thickness). After being dried at 50°C in an oven, the ten leaves mentioned above were weighed dry.

Further, the formula was used to calculate this. The chlorophyll fractions were extracted by the DMSO method [6] from the fresh leaf samples.

2.5 Antioxidant and biochemical estimations

Fully expanded leaves (3^{rd} pair of the leaf from primary branch) samples have been preserved in liquid nitrogen along with kept at -80°C. For the extraction of enzymes, one gram of leaf samples was excised and homogenized in 10millilitres of potassium phosphate buffer (0.1M, pH7.0) comprising 0.2mmol L^{-1} ascorbate and 1% PVPP using a pre-chilled mortar and pestle. The homogenized tissue was subjected to centrifugation at 10,000rpm for 30minutes at 4°C. The supernatant has been harvested and utilized for enzyme tests. The activity of ascorbate peroxidase was assessed by measuring the reduction in ascorbate concentration (ε=2.8mM^{-1}cm^{-1}) during its oxidation [22]. A 3ml reaction mixture was formulated with the following components: 0.5mM ascorbic acid, 0.1mM EDTA, 1.5mM H_2O_2, potassium phosphate buffer (50mM, pH7.0), and 0.1 ml of enzyme. The addition of hydrogen peroxide (H_2O_2) started the process. A Shimadzu UV-1900i UV-visible spectrophotometer was used to measure absorbance at 290nm. The rate at which hydrogen peroxide (H_2O_2) vanishes (ε = 39.4 mM^{-1}cm^{-1}) was used to measure the activity of the enzyme catalase (CAT) [1]. The [14] protocol was followed to determine the epicuticular wax content. Using the [25] approach, the total carbohydrate content was estimated.

2.6 Statistical data analysis

CRBD analysis was used to examine the data, following the methodology pointed out by [27]. The CD values are stated at the 0.01 and 0.05 probability levels. When needed, histographs were used to illustrate the data to identify genotypes that are resistant to drought.

3. RESULTS AND DISCUSSION

The soil moisture depletion was assessed for two years periodically from November to May month to understand soil moisture content (summer season) in the experimental blocks. The soil water deficit during the experiment showed an increasing trend. At initial condition, the soil water deficit was 14.48 mm, and after one month, the soil moisture deficit was increased to 31.02 mm, which was near 50 per cent field capacity (Fig 1). The two years average variation of physiological and biochemical traits is discussed hereunder and presented in figures.

3.1 Gas Exchange Rates

The eight genotypes were studied for carbon exchange parameters. The genotypes significantly differed concerning Intercellular net photosynthesis (Pn),carbon dioxide concentration (Ci), stomatal conductance (gs), respiration rate (E), and and data were presented in (Fig 2). The soil water deficit was 23.96 mm during the observations.

Among the genotypes, increased net photosynthesis, stomatal conductance, transpiration rate and intercellular carbon dioxide were recorded significantly higher ($p<0.05$) in S.4820 (8.04 µmol $m^{-2}sec^{-1}$), Sln.9 and S.4820 (0.045 mol $m^{-2}sec^{-1}$ and 1.01 and 1.00 mmol $m^{-2}sec^{-1}$) and S.5817 (269.93 µl litre^{-1}) respectively. The last gas exchange parameters like net photosynthesis (4.83 µmolm^{-2}sec^{-1}), stomatal conductance (0.022 molm^{-2}sec^{-1}), transpiration rate (0.50) and intercellular carbon dioxide (62.21 µl litre^{-1}) were recorded in S.4202.

The intrinsic mechanisms involving morphological, anatomical, physiological and biochemical characteristics regulate or promote various phases of growth and development besides inducing pest, disease and drought resistance [19]. The physiological mechanism most susceptible to heat stress and drought is photosynthesis. Heat stress can also impede the evolution of oxygen and PSII activity, making photosystem II (PSII) extremely thermo-liable [3; 12]. In the current investigation, it was found that all eight genotypes differed significantly ($p>0.05$) concerning gas exchange parameters. These results coincide with Chlorophyll content and also enzyme activities indicating that the higher CER genotypes have better tolerance to various stresses.

The genotypes significantly differed for carboxylation efficiency (CE), instantaneous as well as intrinsic water utilization efficacy. However, mesophyll effectiveness showed non-significant differences among the genotypes (Fig 3). S.4813 (0.745 μmol m^{-2} sec^{-1} (μl $litre^{-1})^{-1}$) showed significantly higher carboxylation efficiency (C.E.). The instantaneous as well as intrinsic water utilization effectiveness have been observed to be higher in S.4904 (9.97 μmol $mmol^{-1}$) followed by S.4202 (9.19 μmol $mmol^{-1}$). The lowest value was observed in Sln.9 (6.42 μmol $mmol^{-1}$) at a sustainable soil water content of 23.96 mm. The carboxylation efficiency (C.E.) was found to be highest in S.4813, whereas mesophyll efficiency was non-significant among the genotypes. Similar genotypic differences for photosynthetic parameters are reported in coffee genotypes and categorized for carbon exchange rate types [11]. Other crops like watermelon also showed a decrease in water utilization effectiveness with greater drought levels, but resistant cultivars maintained intrinsic water use efficiency because of their better photosynthetic rate during moisture stress [23].

3.2 Growth analysis Traits

The growth analysis traits such as leaf area, SLA, SLW, relative water content, epicuticular wax content and carbohydrate status were determined in different Arabica genotypes and data presented in Fig 4. The genotypes significantly differed concerning leaf area, SLA and SLW. The leaf area, specific leaf area recorded, had been notably ($p<0.05$) higher in S.4904, followed by Sln.9. The lowest leaf area was recorded in S.4808. The Specific leaf weight was significantly higher in Sln.9, followed by S.4813 and S.4202 SLW, reduced during the drought in comparison to the other hybrids. Variations in photosynthetic efficiency and other physiological activity of the distinct genotypes may be connected with variations in SLW. Given that environmental factors (such as dryness) can have a significant impact on leaf area, which is the primary parameter influencing photosynthetic activity in plants, this can serve as a useful criterion for genotype selection. In particular, during the early phases of plant growth, when the leaf area index is the primary factor limiting agricultural photosynthesis, the significance of SLA would be highlighted in the event of stress from drought. SLA can be utilized for screening between genotypes since some genotypes differ in this regard [11]. A genotype's higher SLW suggests improved stress tolerance.

The stress tolerance indicative parameters like relative water content (RWC), epicuticular wax content (ECW) and carbohydrate reserve like total carbohydrate content were also determined in all the genotypes and data presented in Fig 4. RWC is used as one of the techniques for evaluating genotypes for tolerance to water stress.

The relative water content was recorded significantly higher in S.4202 (83.4), followed by S.4820. The genotypic variations in RWC in coffee seedlings and rootstock scion combinations were studied and wide variations were reported at different soil water regimes [9; 33]. In the present investigation, no significant difference was noted for RWC among the hybrids, although higher RWC was observed in S.4820 (80.47%). In the present research, a comparative genotypic difference was noticed in RWC content. Water saving is greatly assisted by ECW, and ECW load is a crucial characteristic linked to drought resistance and physiological efficiency in mulberry plants. ECW is the outermost layer of the leaf, playing an important role in plants' ability to withstand water deficit [10]. Increased wax deposition in drought-resistant cereals [4] has been reported. In considering this, an effort were made in the current research to assess the ECW as one of the criteria for determining whether coffee cultivars are resistant to drought. Coffee plants showed a greater ECW content and a lessened RWC reduction during the soil moisture stress phase [5]. Both arabica and robusta plants have been shown to exhibit an increase in ECW throughout the summer and a decrease during the monsoon season. As a result, this feature may be crucial for determining the capacity of coffee plants to withstand drought [13; 20]. The influence of higher ECW on grain yield was reported in Sorghum. The epicuticular wax content during stress conditions was found to be highest in S.4814.

The carbohydrate content was discovered to be crucial for growth, leaf retention and flowering in coffee [15; 28]. In the current research, the total carbohydrate content differed significantly among the hybrids studied. The genotypes varied significantly ($p<0.05$) in higher carbohydrate content have been noted in Sln.9, followed by S.4820, whereas the lowest carbohydrate content was observed in S.5817. Carbohydrate content was also found to be good in S.4820 (6.07%). Genotypic differences in total carbohydrate content were reported in coffee seedlings [33]. Similar genetic variability concerning carbohydrates was also reported in annual crops such as rice [34]. Based on the utilization of reserve carbohydrate content in different stress periods, coffee genotypes could be classified as better assimilation types.

3.3 Biochemical Parameters

Abiotic stress causes a series of damages through the generation of ROS (reactive oxygen species) in many plant processes comprising degradation of chloroplasts, decrease in chlorophyll concentration, reduction in photosynthesis, and peroxidation of membrane lipids, finally causing the reduction in yield [33; 18; 21]. Oxidative stress is generally measured in terms of both “free radical (O_2^{-}, superoxide radicals; $O.H.^{-}$, hydroxyl radical; HO_2^{-}, per hydroxy radical and $R.O.^{-}$, alkoxy radicals) and non-radical (molecular) forms (H_2O_2, hydrogen peroxide and O_2^{-}, singlet oxygen). Studies were carried out on scavenging enzymes such as Catalase activity, ascorbate peroxidase” activity, and chlorophyll (*a*, *b* & total chlorophyll) content in eight hybrid genotypes and data depicted in Fig 5. The Catalase and ascorbate activity was higher in S.4813, followed by S.4820 and S.4814. Plants adopt various physiological and biochemical mechanisms to cope with changing environments. To combat the effects of ROS produced during stress, plants have developed an inherent mechanism of producing various antioxidant enzymes like Catalase, peroxidase, superoxide dismutase, etc. It has been widely reported that antioxidant enzymes provide tolerance to a variety of abiotic stressors [26].

In the current research, the levels of two antioxidants (Catalase and Ascorbate Peroxidase) were analyzed in the elite hybrids, and it was found that S.4813 (1.0µmol min^{-1}g^{-1} fresh weight, 18.40µmolmin^{-1}g^{-1} fresh weight) and Sln.9 (0.98µmolmin^{-1}g^{-1} fresh weight, 20.90µmolmin^{-1}g^{-1} fresh weight) varieties showed highest catalase and ascorbate Peroxidase activity which indicated that both these arabica hybrids can perform better under various abiotic stresses compare to the other six hybrids studied.

The chlorophyll is the site of photosynthesis. Wide variations in chlorophyll content have been reported among spices and varieties of different crops [17]. In the present study, assessment of chlorophyll fractions *viz.,* Chl`*a*', Chl`*b*' as well as total Chlorophyll (Chl`*c*') was carried out in the selected elite eight promising arabica coffee hybrids in comparison with station-released popular cultivar such as Sln.9. The chlorophyll content such as Chlorophyll '*a*', Chlorophyll '*b*' and total chlorophyll was higher in S.4813, S.5817and S.4820. Significant variations were observed for chlorophyll content between the genotypes. Such genotypic variations for chlorophyll content and photosynthetic efficiency in coffee seedlings were observed [30]. Higher Chlorophyll `*b*' content in coffee was found to increase under abiotic stresses, which could be advantageous in improving photosynthetic efficiency, as positive correlation between total chlorophyll and net photosynthesis was observed [30]. Similar genotypic variations in chlorophyll fractions were reported in coffee. [29] reported that there is a difference in chlorophyll content between the two coffee cultivars, Bourbon (N. 39) and a Kent type (KP 162). [2] reported the existence of variations in the chlorophyll content of eight coffee cultivars.

4. CONCLUSION

Plants are observed to show some intrinsic adaptive mechanisms involving morphological, physiological and biochemical characters that regulate or promote various phases of growth and development besides inducing pest, disease and drought resistance during the peak summer season. Among the genotypes studied, S.4813, S.4814 and S.4820 recorded maximum gas exchange parameters, leaf growth traits and biochemical characters at all the soil moisture deficits compared to other hybrids evaluated. These parameters could prove to be better genotyped with higher stress adaptability. Hence, under the present climate change scenario, these hybrids with increased potential for better yield under abiotic stress could be used in future breeding programme.

5. ARTICLE TYPE

This manuscript is a **Research Article** based on a field study evaluating the new elite arabica coffee hybrids under different moisture regimes.

6. AUTHOR CONTRIBUTION

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Somashekhargouda Patil, Yashaswini K P, Yashasvi H R, Jeena Devasia and M. Senthilkumar. The first draft of the manuscript was written by Somashekhargouda Patil, and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the" manuscript.

7. CONFLICT "OF INTEREST

The authors declare that they have no conflicts of interest.

8. ACKNOWLEDGMENT

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Fig 1: Mean monthly variation of soil moisture deficit (mm) in the experimental site

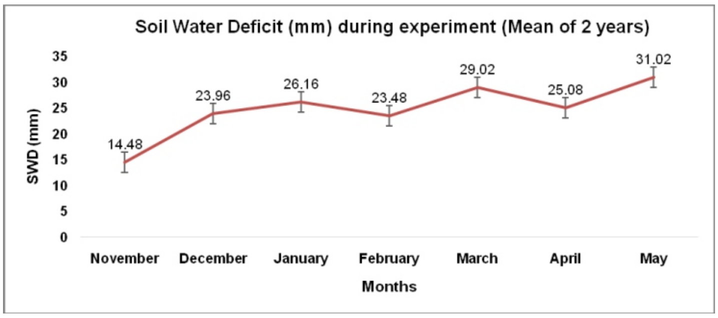
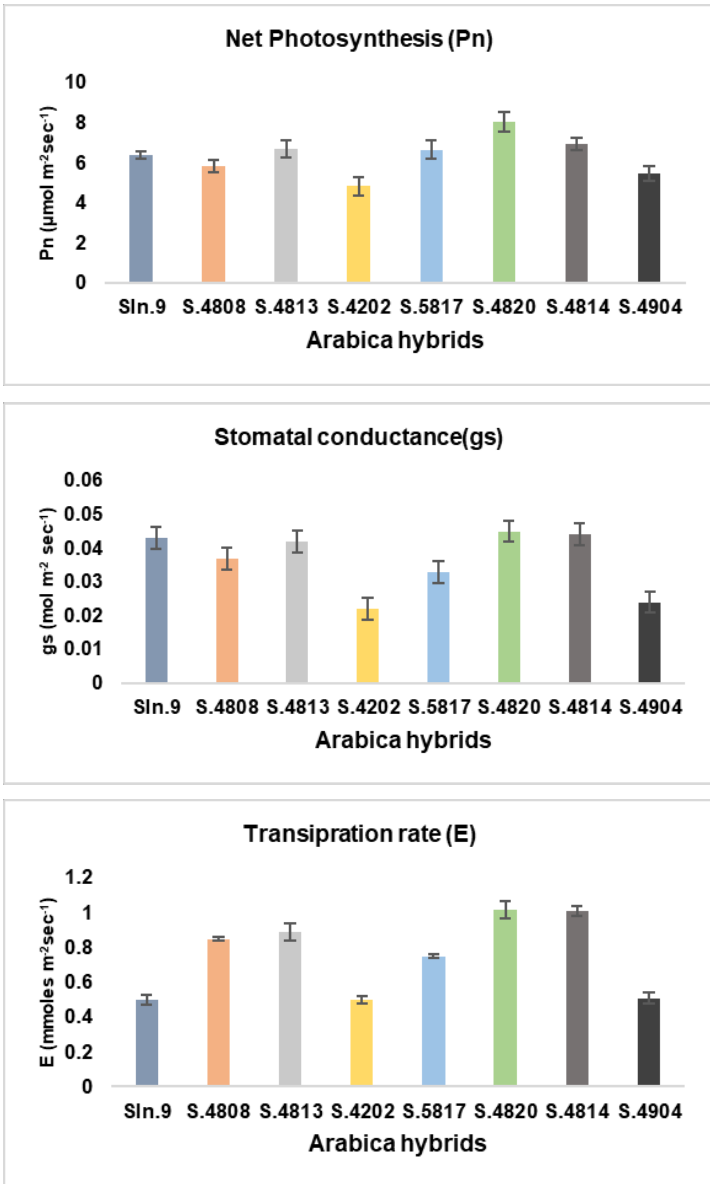


Fig 2: Genetic variation of carbon exchange rates in elite Arabica coffee hybrids



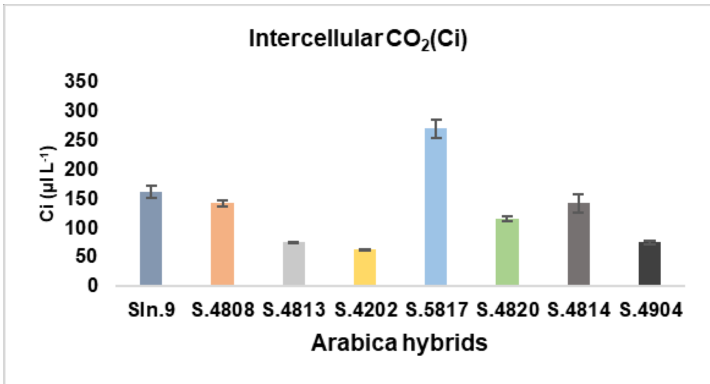
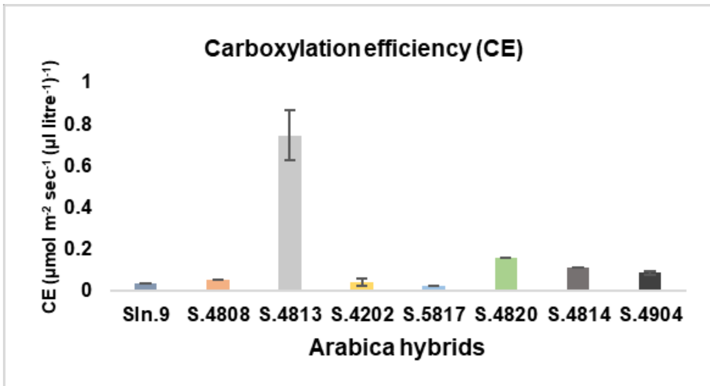
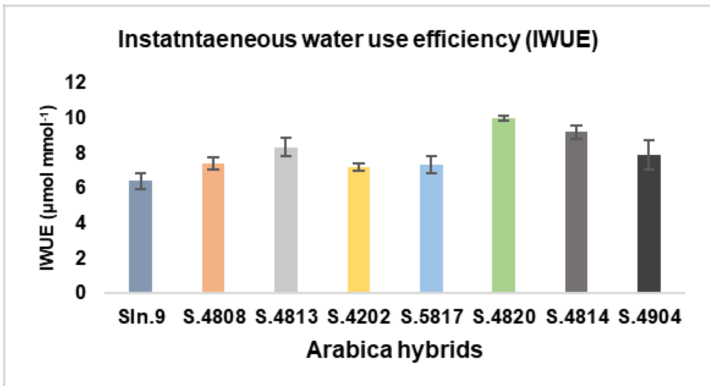
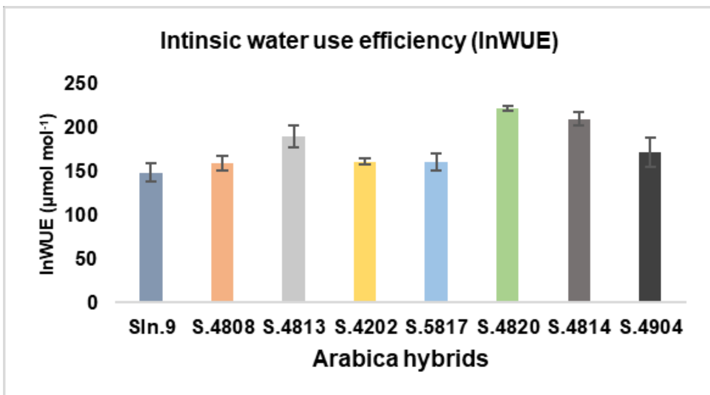


Fig 3: Genetic variation on carboxylation efficiency, instantaeneous water use efficiency, intrinsic water use efficiency and mesophyll efficiency in elite Arabica coffee hybrids







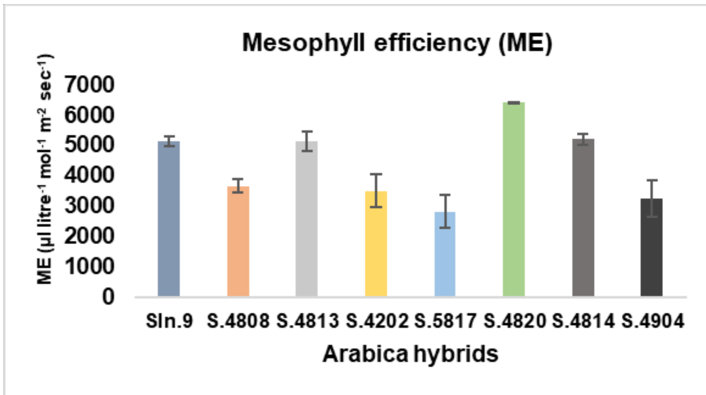
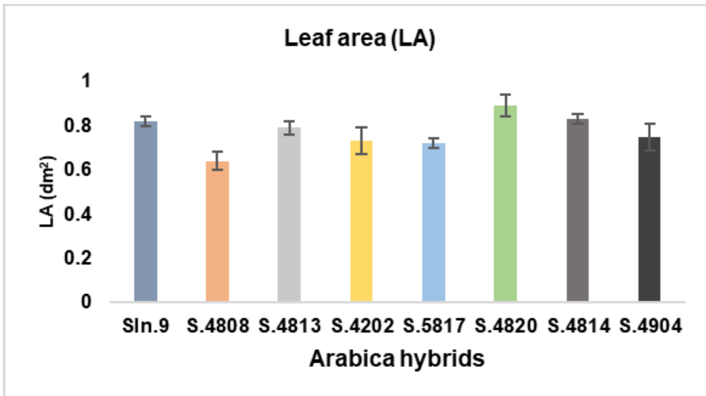
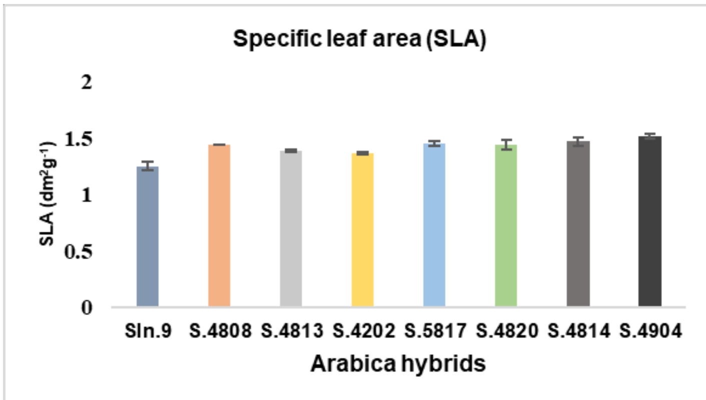
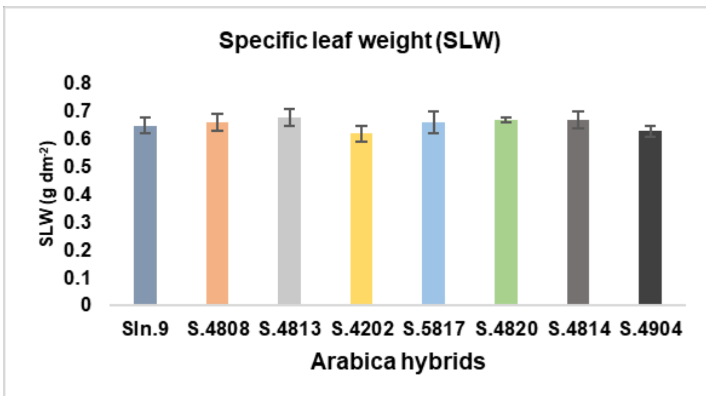


Fig 4: Genetic variation on leaf growth parameters in elite Arabica coffee hybrids







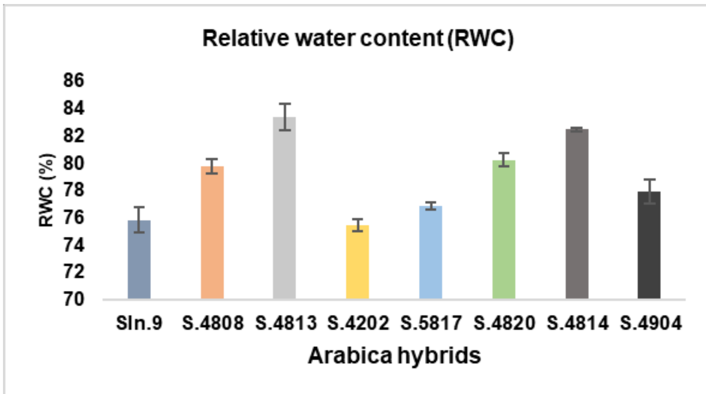
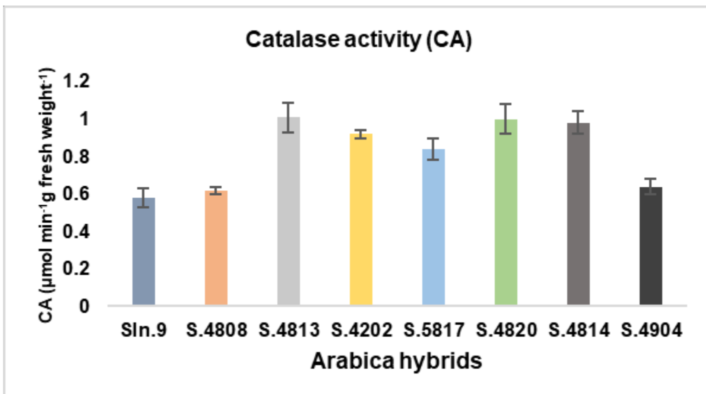
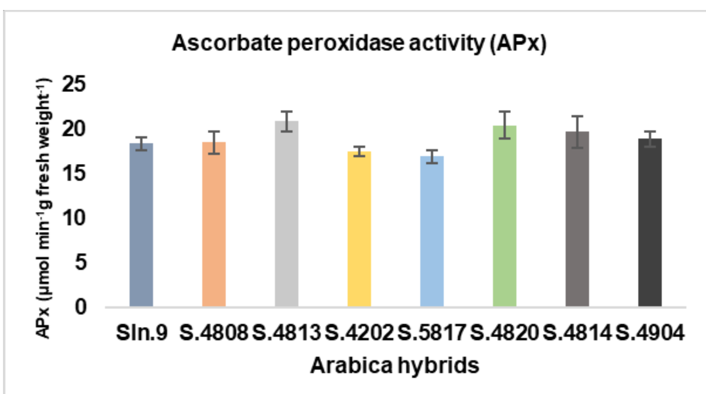
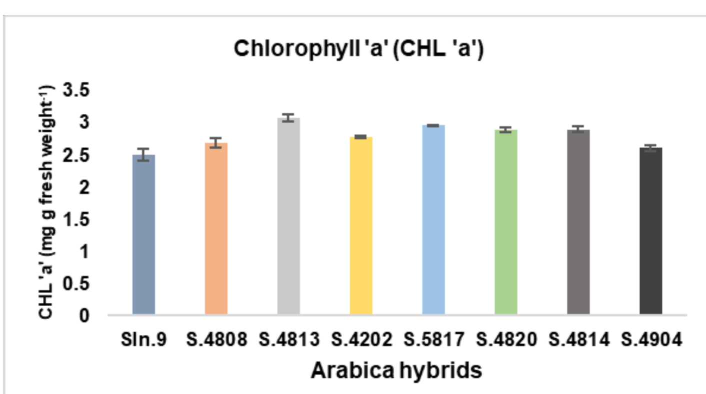
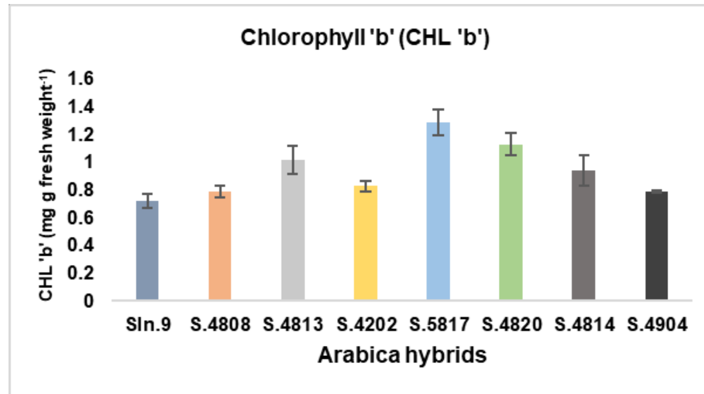


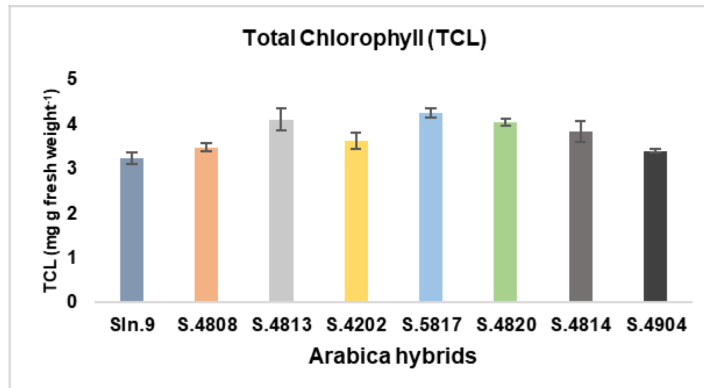
Fig 5: Genetic variation on biochemical parameters in elite Arabica coffee hybrids











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