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Physiology of biotic and abiotic stress tolerance in agricultural crops with special reference to tea, *Camellia sinensis* (L.) O. Kuntze

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Abstract

Ascertaining the sustainability of current status and future food security is considered as the topmost challenge in agricultural crop production. Approximately 90% of arable lands are prone to one or more such stresses, which accounts for about 70% of the loss of different food crops. The climate is changing frequently and with the changing climate, plants are subjected to different abiotic and biotic stresses like drought, diseases, and pests. The tolerance level of six selected tea clones was evaluated against drought, dieback disease, one chewing pest, namely looper (*Hyposidra talaca*) and one sucking pest, namely tea mosquito bug (*Helopeltis theivora*). The results indicated that clone 107/17 performed superior physiologically and biochemically among the assessed clones. For physiological evaluation, the portable photosynthetic meter LI-COR 6400 XT was used. In looper susceptibility evaluation, clone 107/17 performed well but failed to show tolerance against the tea mosquito bug. The other two promising clones performed at par with the two control clones, TV1 and TV18.

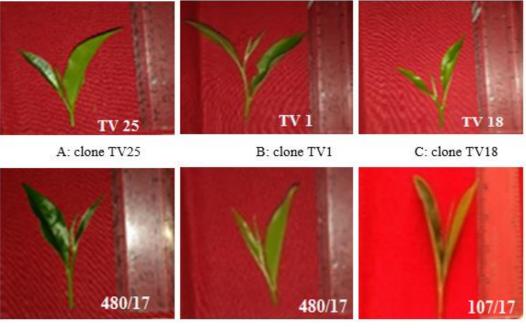
Keywords: tea, clone, climate, drought, pest, disease, proline, wax, susceptibility, tolerance

Introduction

Both abiotic and biotic stress in plants plays a significant role in today's agriculture. Stress can be abiotic, like drought, water logging, high and low temperatures, changes in soil conditions, etc., and can also be biotic, like different pests and diseases ^[1]. These stresses not only harm the nature of plant growth but also reduce crop production, which may be leaves, fruit, or flowers. Tea is a perennial commercial beverage crop that is long-living and capable of yielding for more than a century when it is managed appropriately ^[1]. Tea requires 4.5-5.5 pH and 2% organic matter is also essential for tea cultivation ^[2]. There are different types of tea like CTC, Orthodox, and Green tea based on the fermentation period. The chemical composition of each differs. In 1997, Kim and Masuda^[3] reported that green tea polyphenols have activities like trapping of carcinogens, nitrosation reactions, inhibition of biochemical signals of tumor initiation, growth of intestinal clostridia, etc. Various research studies have proved that tea has many different mechanisms to display a protective role against cardiovascular diseases. The changing climate presents plant diseases and pests which are the foremost threat to worldwide crop production and food security. Tea is like any other perennial crop which is affected by many pests ^[4]. There are more than a thousand arthropod tea pests which cause about 5-55% of yield loss ^[5]. Hyposidra talaca Walker is a lepidopteron pest migrated to tea plantations of North Eastern India from nearby forest areas dominated by Sal trees during the year 2008-09 and nowadays a major concern for planters of the Dooars, Terai, and Assam tea industries [1]. Helopeltis theivora Waterhouse is a sucking pest also considered a major pest of tea plantations in India and is responsible for about 10-50% of yield loss ^[4]. Dieback is a secondary disease of tea which is caused by a fungus named Fusarium solani, which accounts for severe damage to crop production ^[6] and is a major concern for the tea industry ^[7]. The productivity of many crops is severely under threat from abiotic and biotic stresses ^[8]. The same planting material creates homozygosity, which becomes favorable for several pests and diseases. Though there are many chemical and biological measures to prevent stress effects, it is necessary to develop stress-tolerant tea clones to develop

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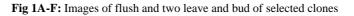
heterozygosity and for the sustainability of the industry. The productivity of many crops is severely under threat of abiotic and biotic stresses. Same planting material creates homozygosity which becomes favorable for several pests and diseases. Though there are many chemical and biological measures to prevent stress effects, it is necessary to develop stress-tolerant tea clones to develop heterozygosity and for the sustainability of the industry.



D: clone480/7

E: clone 480/17

F: clone 107/17



Materials and Methods

Physiological Assessment of selected clones

Six different clones, *viz.*, TV 25, TV1, TV 18, 480/7, 480/17 and 107/17 were selected based on the quality and yield from the Dooars region, West Bengal, India (Fig 1). The study was carried out using LI-COR 6400XT [http://www.licor.com (US Patent 5332901 & 5340987)]. Different parameters were adjusted as per the standard protocol *viz.*, Photosynthetic rate: μ mol CO₂ m⁻²s⁻¹, Transpiration rate: mmol H₂O m⁻²s⁻¹, Water use efficiency: μ mol CO₂ m⁻²s⁻¹, Photosynthetic active radiation: μ mol m⁻²s⁻¹, Atmospheric pressure: kPa, CO2 concentration: μ mol CO₂ mol⁻¹, Vapour pressure deficit: kPa, Stomatal conductance: mol H₂O m⁻²s⁻¹, leaf area (surface area of sensor): 6 cm², and leaf temperature: ⁰C.

Anatomical assessment of selected clones

The leaves and nodal parts (same age) of each clone were collected for study. T.S. (Transverse section) was done on each sample for leaf and nodal anatomy by staining with 1% safranin solution. Images were observed under an Olympus CX 21i microscope and measurements were done through the Magvision application at 10, 40, and 100 X magnification. For stomata, leaves were peeled off with a blade and stomata were observed under the same microscope and the stomatal index of each clone was counted.

Die Back Susceptibility

The study was carried out by following the methodology of ^[6]. Ten numbers of bushes were selected from each replication. Bushes are marked and searched for infection by *Fusarium solani* (Die-back disease). Infected plant parts are collected from the field and cultured in PDA (Potato Dextrose Agar) media to confirm the causal organism. Cultured plates were monitored for 1 or 2 weeks to check the same pattern of

growth caused by direct spores and infected plant parts. Microscopic slides of the fungal growth were prepared and observed under an Olympus compound microscope (Magcam DC 10 camera). The same procedure for field experiments continued for a full year (at a monthly interval) not only to determine which clone is tolerant or susceptible to the disease, but also the peak susceptible season of die-back disease.

Looper (Hyposidra talaca) susceptibility

The study was carried out by following the methodology of Gurusubramanian and Bora (2007) ^[22]. Loopers were collected from the field and reared in the entomological laboratory of TRA, NBRRDC, Nagrakata till the next generation. Fresh shoots were collected from the experimental plot and weighed. Shoots are placed in the separate vowels under a chimney. A single looper is given to shoots of each clone along with leaving a shoot for control (with water) and a shoot for transpiration (without water). The next day, the shoots were weighed and recorded. The same procedure followed on the third day and accordingly the analysis for determination of feeding preference was carried out ^[22].

Tea mosquito bug (Helopeltis theivora) susceptibility

The study was carried out under laboratory conditions by following the methodology of Roy *et al.*, (2009) ^[21]. Fresh shoots of each clone were collected from the field during plucking and 3 shoots of each replication were put into a vial within a wooden cage. Three numbers of tea mosquito bugs reared under laboratory conditions were released in that particular cage. After 24hrs, the leaf punctures of each clone were counted. Under field conditions, after plucking, 100 plucked shoots of each clone were collected for study from each replication. Shoots fed by *Helopeltis theivora were* counted and documented and, accordingly, the percentage of

infestation was calculated for statistical analysis (Gurusubramanian and Bora, 2007)^[22].

Results and Discussion

Crop record (KMTH) six assessed clones

In 2016, the highest crop was produced by clone 107/17, followed by TV25 and clone 480/7, which is statistically

significant ($P \le 0.05$) with all the clones except TV25. In 2017, the highest crop was produced by clone 107/17, followed by TV25 and clone 480/17, which is statistically significant ($P \le 0.05$) with all the clones except TV25. In 2018, the highest crop was produced by clone 107/17, followed by TV25 and clone 480/7, which is statistically significant ($P \le 0.05$) among all the assessed clones (Table 1).

Clone	Yield (KMTH) (Made tea per hectare)					
	2016	2017	2018			
480/7*	2301.69	1481.24	1261.25			
480/17*	2297.37	1478.19	1198.36			
TV25**	2621.53	1988.85	1492.34			
TV1**	1773.44	1526.48	1100.83			
107/17*	3121.30	2451.31	1851.79			
TV18**	2076.13	1500.67	1118.56			
CD	440.31	461.47	283.21			
CV	10.10	14.41	11.49			

 Table 1: Assessment of yield among experimented clones

The photosynthetic rate varies between 19.69 and 21.90. Among all selected clones, 107/17 showed the highest photosynthesis (Pn) rate followed by TV25 and 480/17. Clone 107/17 showed significant variation ($P \le 0.05$) with all the assessed clones except TV25. The transpiration rate varies between 5.62-6.03 µmolH20m⁻²s⁻¹. Among all selected clones, 107/17 showed the highest transpiration rate, followed by TV25 and TV18. Clone 107/17 showed significant variation ($P \leq 0.05$) with all the assessed clones. Water use efficiency varies between 3.47 - 3.81 µmol CO2 m⁻²s⁻¹. Among all selected clones, TV25 showed significant ($P \leq 0.05$) higher water use efficiency than others, except clone 480/17, followed by 480/17 and 107/17. The leaf temperature varies between 26.16 and 27.17 ^oC among all selected clones. 107/17 showed the highest leaf temperature followed by TV25 and 480/17. Clone 107/17 showed significant variation $(P \leq 0.05)$ with TV18 and Clone 480/7. The CO2 concentration varies between 0.291 and 0.406 µmol CO₂ mol⁻¹. Among all

selected clones, TV25showed the highest CO2 concentration, which significantly varied ($P \le 0.05$) from others, except clone 107/17, followed by 107/17 and 480/17. The photosynthetic active reaction varies between 882.04 and 1037.64 µmol m⁻²s-¹. Among all selected clones, 107/17 showed the highest PAR rate followed by 480/17 and TV25, which significantly varied $(P \leq 0.05)$ with other assessed clones except clones 480/17 and TV25. The vapour pressure deficit varies between 2.35 and 2.68. Clone 107/17 showed the lowest vapour pressure deficit which insignificantly varied ($P \leq 0.05$) with others except TV1, followed by TV18 and clone 480/7. The relative humidity varies between 63.58 and 64.66%. Among all selected clones, TV25 had the highest relative humidity followed by clone 107/17, which insignificantly ($P \leq 0.05$) among themselves. Stomatal conductance varies between 0.251 and 0.291. Stomatal conductance was lowest in TV25, which is statistically significant ($P \leq 0.05$) than in other clones except clone 107/17, followed by clone 480/17 (Table 2).

Table 2: F	Physiological	evaluation	of si	ix clones
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Clone	Photosynthetic rate (µmol CO ₂ m ⁻ ² s ⁻¹)	Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)	Water use efficiency (µmol CO2m ⁻² s ⁻¹)	Leaf Temperature (0C)	CO ₂ Concentration (Ci/Ca)	PAR (Photosynthetic active reaction) (μmol m-2s-1)	Vapour pressure deficit (kPa)	Relative humidity (%)	Stomatal conductance (mol m ⁻² s ⁻¹)
480/17	20.70±0.326	5.62 ± 0.056	3.70 ± 0.092	26.42 ± 0.500	0.361±0.009	1003.05 ± 25.015	2.53 ± 0.027	63.60±0.941	0.261 ± 0.004
480/7	19.97±0.351	5.60±0.073	3.58 ± 0.028	26.57±0.291	0.340±0.013	942.75±20.269	2.45 ± 0.083	63.95±0.771	0.291±0.013
107/17	21.90±0.461	6.03±0.151	3.68 ± 0.040	27.17±0.491	0.387±0.014	1037.64±21.733	2.35±0.133	64.66 ± 0.998	0.266 ± 0.015
TV1	20.24±0.475	5.70 ± 0.038	3.56 ± 0.056	26.37±0.206	0.309±0.011	882.04±7.992	2.68±0.151	63.58±0.259	0.283 ± 0.020
TV25	21.74±0.191	5.75 ± 0.065	3.81 ± 0.037	26.89±0.309	0.406 ± 0.009	985.93±10.433	2.50±0.189	64.67 ± 0.262	0.251 ± 0.011
TV18	19.69±0.623	5.71±0.042	3.47 ± 0.109	26.16±0.618	0.291±0.008	958.08±37.975	2.43 ± 0.066	63.86±0.174	0.281 ± 0.008
CD	0.61	0.12	0.13	0.11	0.017	44.38	0.18	N/A	0.025
CV	1.61	1.10	1.91	1.45	2.594	2.49	3.89	0.89	5.043

Values represent average with standard deviation (SD) of three replications.* represents promising tea clones in Tea Research Association, Nagrakata taken for study. ** represents released and well-established varieties in Dooars agro-climatic zone. TV1 was used as a quality clone, TV18 as a yield clone, and TV25 as drought-tolerant clone.

Anatomical assessment of selected clones A. Leaf

[Evaluation of thickness of epidermis and length and breadth of palisade parenchyma (Unit: micrometers)]. The first mature leaf of uniform age of all the tea clones was used for the assessment (Fig 2A). Among all selected clones, 480/17 showed the highest thickness of leaf epidermis, which showed significant variation ($P \le 0.05$) with all the clones except 107/17, followed by 107/17 and TV25 throughout 2018. TV25 showed Palisade's highest parenchyma length (leaf) which showed significant variation ($P \le 0.05$) with all the clones except clone 107/17, followed by 107/17 and TV18 throughout 2018. Clone 480/17 showed the highest Palisade parenchyma breadth (leaf) which showed insignificant variation ($P \le 0.05$) with clone 107/17 and clone 480/7 followed by 107/17 and 480/7 throughout 2018 (Table 3).

Clone	107/17*	480/7*	480/17*	TV1**	TV18**	TV25**
Palisade Parenchyma(L)	825.57	624.18	740.15	685.17	747.31	839.53
SD (N = 4)	55.67	56.58	61.91	50.34	58.54	64.14
Palisade Parenchyma(B)	170.77	163.93	173.82	153.62	143.34	152.90
SD (N = 4)	8.36	8.85	9.87	10.47	5.55	5.51
Thickness Of Epidermis	280.14	242.42	288.86	245.48	211.79	266.15
SD (N = 4)	11.89	11.68	13.89	10.70	10.03	13.57

Table 3: Morphometric assessment of leaf

B. Nodal Anatomy: [Evaluation of Epidermis, Collenchyma, Cortex, Sclerenchyma, Phloem, and Xylem (Unit: micrometer)]: Node of uniform age of all the tea clones used for the assessment (Fig 2B). Among all selected clones, 107/17 showed the highest thickness of the epidermis (Node) which showed significant variation ($P \le 0.05$) with all the clones except clone 480/7, followed by 480/7 and 480/17 throughout 2018. Clone 107/17 showed the highest thickness of collenchyma (Node) which showed significant variation ($P \le 0.05$) among all the clones followed by TV25 and TV18 throughout 2018. Clone 107/17 showed the highest thickness of cortex (Node) which showed significant variation ($P \le 0.05$)

among all the clones followed by TV25 and TV18 throughout 2018. Clone 107/17 showed the highest sclerenchyma (Node) thickness which showed significant variation ($P \le 0.05$) among all the clones followed by TV25 and TV1 throughout 2018. Clone 107/17 showed the highest thickness of phloem (Node) which showed significant variation ($P \le 0.05$) with all the clones except TV18, followed by TV25 and 480/17 throughout 2018. Clone 107/17 showed the highest thickness of xylem (Node) which showed significant variation ($P \le 0.05$) with all the clones except TV18, followed by TV25 and TV18 throughout 2018 (Table 4).

Table 4: Morphometric assessment of Node

Clone	Epidermis (μm) (N=4)	Collenchyma (µm)(N = 4)	Cortex (µm) (N=4)	Sclerenchyma (µm)(N = 4)	Phloem (µm) (N=4)	Xylem (µm) (N=4)
480/7*	28.56	33.34	200.71	60.46	90.25	19.69
480/17*	27.08	31.99	220.62	61.17	90.40	20.10
107/17*	28.96	36.52	275.82	90.92	101.14	22.00
TV1**	23.94	30.36	216.25	71.70	84.56	20.31
TV25**	24.48	34.19	250.24	83.20	98.27	21.95
TV18**	22.17	33.42	221.01	66.79	89.85	20.67

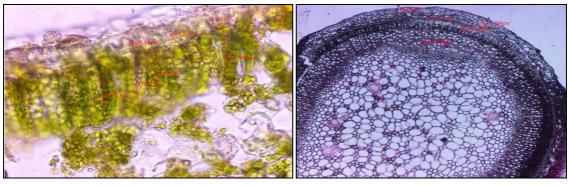
C. Stomata: (Dimension of guard cell and aperture in micrometers)

Middle part of leaf: First mature leaf of uniform age of all the tea clones used for the assessment (Fig 3A and B). Among all selected clones, clone 107/17 showed the significantly ($P \leq 0.05$) highest guard cell length followed by TV25. Clone

480/17showed the highest guard cell breadth. In the case of aperture, clone 107/17 insignificantly ($P \le 0.05$) showed the highest length, and TV18 showed the thickest breadth. Clone 480/17 exhibited the lowest thickness in aperture breadth (Table 5).

Table 5: Morphometric assessmen	nt of stomata from base of the leaf
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	BASE							
Clone	Guard cell length (N = 4)	Guard cell breadth (N = 4)	Aperture length (N = 4)	Aperture breadth (N = 4)				
TV18*	823.27	271.94	306.26	38.37				
TV25*	944.24	281.05	285.05	29.34				
TV1*	852.64	270.62	309.83	34.02				
107/17**	945.14	286.74	354.20	34.81				
480/7**	908.27	267.04	284.28	34.74				
480/17**	928.27	310.55	288.81	25.78				



А

В

Fig 2: Images of Leaf and Stem anatomy [scale: 300µm]. A: leaf anatomy Image, B: Nodal anatomy

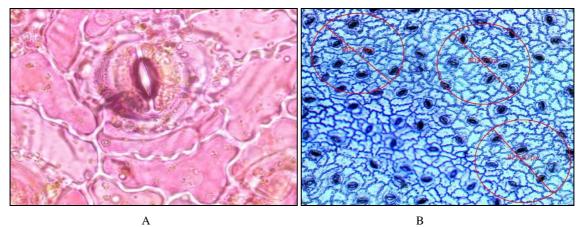


Fig 3: Images of stomatal measurement [scale: 250µm]. A: Stomata under 100X magnification, B: Measurement of stomatal index

Feeding Susceptibility against H. theivora

For feeding behavior of *H. theivora*, TV1 is used as a quality clone, TV18 as a yield clone, and TV25 as a drought-tolerant clone. It was observed that among the clones, 107/17 had the highest feeding punctures and TV 25 had the lowest. According to the host preference of the pest, the clones under study can be arranged in descending order as: Clone 107/17 > TV1 > Clone 480/17 > Clone 480/7 > TV18 > TV25. Statistical analysis revealed that among different clones. TV 25 showed significant variation ($P \leq 0.05$) with all of the control clones except TV18 and 480/7 (Fig 4).

For percent infestation of H. theivora study, TV1 is used as a

quality clone, TV18 as a yield clone, and TV25 as a droughttolerant clone. A similar pattern of observations was obtained in laboratory-based and field-based assessments of the concerned pest. The results showed that among the clones, 107/17 (56.59, SD=6.52) had the highest feeding punctures and TV 25 (24.71, SD=4.62) had the lowest. Among promising clones, 480/7 showed lower feeding preference. Statistically, significant variation was found among different clones. TV 25 showed significant variation ($P \le 0.5$) with all of the clones except TV18 and 480/7. Therefore, the clone TV25 is least preferred by the pest, and the clone 107/17 is most preferred by the pest (Fig 5).

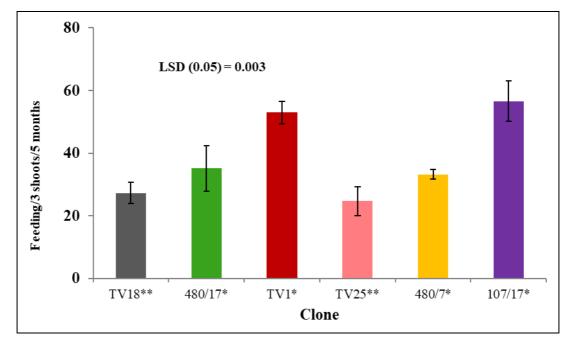


Fig 4: Histogram showing evaluation of feeding behavior of *H. theivora* on selected tea clones. * represents promising tea clones in Tea Research Association, Nagrakata. Values represent average with standard deviation (SD) of five replications. ** represents Tocklai released and well-established varieties under Dooars agro-climatic zone.

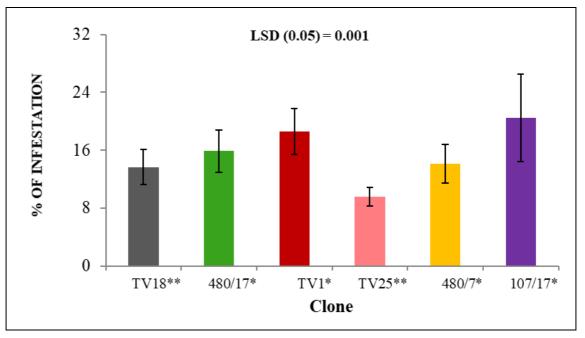


Fig 5: Histogram showing evaluation of feeding behavior of *H. theivora* on selected tea clones. * represents promising tea clones in Tea Research Association, Nagrakata. Values represent average with standard deviation (SD) of five replications. ** represents Tocklai released and well-established varieties under Dooars agro-climatic zone

Feeding Susceptibility against H. talaca

The feeding value varies between 3.85 to 6.12 mg/second instar looper between different clones. Promising clone 107/17 showed the least feeding (3.85 mg, SD=0.33) and also showed significant variation ($P \leq 0.5$) with all other assessed clones, followed by TV25 (4.42 mg, SD=0.34) and clone

480/17 (4.48 mg, SD=0.40). The feeding value was highest in TV1 (6.12 mg, SD=0.25), which showed significant variation among all the experimented clones. Therefore, the clone 107/17 is least preferred by the pest, and the clone TV1 is most preferred by the pest (Fig 6).

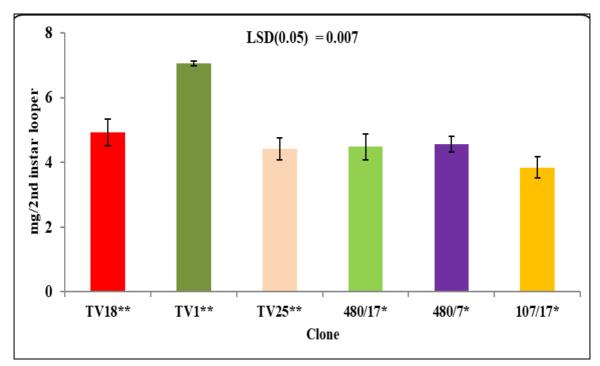


Fig 6: Bar diagram representing the evaluation of feeding behavior, in terms of quantity of green leaf, of *H. talaca* 2nd instar on different clones in Dooars agro-climatic condition. Values represent average with standard deviation (SD) of four replications.

* represents promising tea clones under trial; ** represents released and well-established varieties in Dooars agro-climatic zone.

Dieback susceptibility

In this study, TV1 was used as a quality clone, TV18 as a yield clone, and TV25 as a drought-tolerant clone. Promising clones are assessed throughout the year for their susceptibility to die-back disease along with their controls. In the study

clone, 107/17 showed the lowest susceptibility, and TV1 showed the highest susceptibility for dieback disease. The variation was statistically significant ($P \leq 0.05$) between clone 107/17 and TV1 (Fig 7).

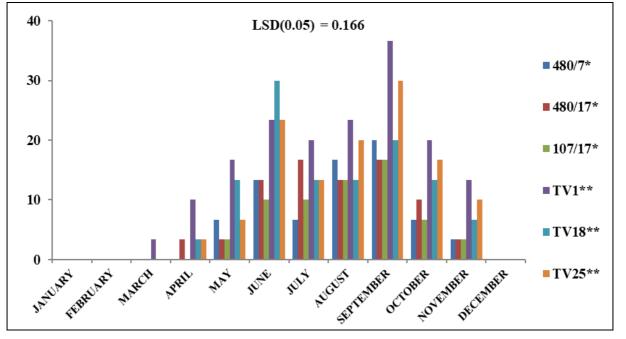


Fig 7: Dieback disease susceptibility experiment of selected tea clones. * represents promising tea clones in Tea Research Association, Nagrakata taken for study. ** represents released and well-established varieties in Dooars agro-climatic zone

This study emphasized the capability of a plant to endure drought stress or dry climatic conditions, either as a consequence of stable climatic characteristics or by an extended spell of dryness in the atmosphere and soil watertable. The efficiency of the pipeline clones in tolerating the drought or endurance is discussed. Enhanced photosynthesis even in a compromised water relationship, lesser availability of water from the soil profile, and decreased moisture level in the ambient atmospheric parameters have played an important role in selecting promising tea clones for propagation. The photosynthetic rate varies between 19.69-21.90 micromol of CO_2 m⁻²s⁻¹. The percentage hike in photosynthetic rate than released check TV18 was recorded at 11.22 in pipeline clone 107/17, which marked the highest score in terms of the said parameter. The released drought-tolerant check was TV25 (with an increment of 10.41) and the pipeline clone as mentioned performed even better than the released cultivar. Another clone 480/17 recorded the value as being 10.21 might be assigned the second rank.

Anatomical morphometry also indicated better adaptability and morpho-anatomical response to drought stress, and the promising clone107/17 performed better in all such parameters. The results showed that among the clones, 107/17 had the highest percentage of infestation by H. theivora and TV 25 had the lowest. Among promising clones, 480/17 showed the lowest percentage of infestation. However, successful pest management could be a practically implemented answer for the tea cultivating region or garden. However, tea is such a crop for which there should be a balanced blend of three attributes: productivity, quality, and tolerance. Successful photosynthesis even in low water availability with better water management physiological efficiency proved to be favorable for high productivity. Secondary metabolite content like carotenoids, flavonoids, alkaloids, which have been variously documented in plant species as an indication of the successful attempt at stress endurance, could be related to the uninterrupted and enhanced photosynthetic and growth-related parameters of the promising clones of tea.

is not additive since the result is usually determined by the type of interactions between the stress factors themselves ^{[11,} ^{12, 13]}. Plants adapt their reactions to a combination of stress conditions, exhibiting a number of distinct responses in addition to other typical responses to stress. As a result, in order to properly comprehend the effect of combined abiotic and biotic stressors on plants, it is necessary to first understand the nature of these interactions themselves ^[14, 15] (Mittler, 2006; Suzuki et al., 2014). Drought, high and low temperatures, and salinity are all known to affect the presence and spread of diseases, insects, and weeds ^[16, 17] (Ziska et al., 2010; Peters et al., 2014). They may also cause minor pests to become potential threats in the future ^[18] (Duveiller et al., 2007). These stresses also have a direct impact on plant-pest interactions by changing plant physiology and defensive responses [19] (Scherm and Coakley, 2003). Furthermore, abiotic stress situations such as drought increase competitive interactions between weeds and crops since many weeds have higher water use efficiency than crops ^[16, 20] (Ziska et al., 2010; Valerio et al., 2013).

Conclusion

The efficiency of the pipeline clones in tolerating the drought or endurance is discussed. The percentage hike in photosynthetic rate than released check TV18 was recorded at 11.22 in pipeline clone 107/17, which marked the highest score in terms of the said parameter. The pipeline clone recorded a percentage increment of 7.68, which was higher than others. The released drought-tolerant check was TV25 and the pipeline clone performed even better than the released cultivar. Clan 107/17 showed the lowest Vapour pressure deficit; Relative humidity varied between 63.58 - 64.66%; Stomatal conductance varied between 0.251 - 0.291; Leaf cuticular waxes play an important role in plant resistance to a variety of biotic and abiotic stresses, such as those caused by fungal pathogens, phytophagous insects, freezing temperatures, and drought. The results showed that among the clones, 107/17 had the highest percentage of infestation by Helopeltis theivora and TV 25 had the lowest.

In most cases, the combined impact of stress factors on crops

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