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Research article

Efficient vegetation indices for phenotyping of abiotic stress tolerance in tea plant (*Camellia sinensis* (L.) Kuntze)

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ABSTRACT

Early non-destructive detection of stress effect is crucial for efficient breeding strategies and germplasm characterization. Recently developed hyperspectral technologies allow to perform fast real-time phenotyping through reflectance-based vegetation indices. However, efficiency of these vegetation indices has to be validated for each crop in different environment. The aim of this study was to reveal efficient vegetation indices for phenotyping of abiotic stress (cold, freezing and nitrogen deficiency) response in tea plant. Among 31 studied VIs, few indices were efficient to distinguish tolerant and susceptible tea plants under abiotic stress: ZMI (Zarco-Tejada & Miller Index), VREI1,2,3 (Vogelmann Red Edge Indices), RENDVI (Red Edge Normalized Difference Vegetation Index), CTR1 and CTR2 (Carter Indices). Most of these indices are calculated based on reflectance in near-infrared area at 705–760 nm, indicating this range as promising for tea germplasm characterization under abiotic stresses. Tolerant tea plants showed the following values under freezing: ZMI $\geq$1.90, VREI1 $\geq$ 1.40, RENDVI $\geq$0.38, Ctr1 $\leq$ 1.74. The leaf N-content was positively correlated (Pearson's) with the following indices ZMI, VREI1, RENDVI, while negatively correlated with CTR, and VREI2,3. These results will be useful for tea germplasm management, genomics and breeding research aimed at abiotic stress tolerance of tea plant.

1. Introduction

Early detection of phenotypic stress responses in plants is important for germplasm characterization and breeding research. However, traditional methods for plant phenotyping are usually time and labor consuming. This makes difficult to fulfill the high-throughput phenotyping in large-scale experiments. In recent years, hyperspectral technologies have been developed as efficient approaches for the non-destructive detection plant health status [1]. Particularly, the reflectance-based spectrometry provides valuable information about the plant growth and responses to stimuli. In the visible bands, the typical leaf reflectance range is just 10–20 %, while in the near-infrared area at 700–1000 nm it is 40–50 %. When leaves are in the growing phase, the red edge inflection point

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shifts toward longer wavelengths, then shifts back to shorter wavelengths when there is a water shortage and leaf discoloration [2]. The measurement of reflectance using handled spectrometers is one the most advantageous method of data acquisition without anisotropy effect [2,3]. Under the abiotic stresses the spectral line shifts as result of biochemical changes which is well described by a wide range of vegetation indices (VIs) [1,4]. Modern portable spectrometers allow to get data in a broad range of spectrum (usually 300–1100 nm), and embedded software calculates different VIs in real time, in about 10 s per measurement.

Up to now, more than hundred VIs were developed, however, not all of them are really efficient and crop-transferable. Among most commonly used indices, three functional groups are presented: first group indicates water status of plants (water band index (WBI), equivalent water thickness (EWT) et al.), second group focused on photosynthetic parameters (photosynthetic rate index (PRI), Normalized Difference Vegetation Index (NDVI), Transformed Chlorophyll Absorption in Reflectance Index (TCARI), Triangular Vegetation Index (TVI) et al.) and the third one is related to secondary metabolism (carotenoids, polyphenols, anthocyanidins) (Anthocyanin Reflectance Index (ANR), Carotenoid Reflectance Index (CRI), Structure Intensive Pigment Index (SIPI) et al.) [5–7]. According to the other classification, all indices can be categorized into four classes: 1) the ratio indices (for example WBI); 2) the normalized difference indices (for example NDVI); 3) the triangular area-based indices (for example TVI); and 4) the integrated indices (for example TCARI) [8].

The efficiency of different indices can depend on the plant species and environmental conditions [9,10]. Thus, it is necessary to evaluate the efficiency of each VI for particular crop in a certain condition for its reliable phenotyping. Usually, reflectance spectrum of the whole foliage is used to derive an average VI. However, the individual leaves of the same plant displayed low dispersion of VIs, for example in oak and beech trees [11]. Thus, handled spot spectrometry not only allow to check accuracy of a certain VIs but also help to reveal the best wavelength area for each crop to develop the more precise and reliable indices for phenotyping.

Tea plant (*Camellia sinensis* (L.) Kuntze) is an important evergreen tree crop grown in more than 60 countries on five continents, from 49°N in Ukraine to 33°S in South Africa [12]. Tea leaves are collected for production of popular non-alcoholic beverage [13,14]. Tea leaf quality depends on the contents of bioactive compounds such as polyphenols, caffeine, and L-theanine, amino acids, volatile compounds, and alkaloids which underly the delicious taste, pleasant flavor and the health beneficial effect [15]. To improve tea quality and tolerance to various stress factors, genome wide association studies is topical research direction worldwide [16]. For the genomic studies, efficient phenotyping is extremely important and challenging in tree crops, particularly in tea crop [17]. Abiotic stresses such as cold, drought and nitrogen deficiency are serious constrains for the world tea industry. Although remote sensing has been widely reported in different crops, few efforts have been made in tea crop [18]. Thus, the aim of this study was to evaluate the efficiency of the most commonly used reflectance-based VIs and spectrum areas for phenotyping of abiotic stress responses in tea plant. In this study, we evaluated the efficiency of 31 reflectance-based VIs, calculated in 300–1100 nm, for phenotyping of abiotic stress response in tea plant.

2. Materials and methods

2.1. Nitrogen deficiency experiment

Plant material: Plants were obtained from the collection of the Federal Research Centre the Subtropical Scientific Centre of the Russian Academy of Sciences (FRC SSC RAS, 43.569975° N, 39.749984° E). For nitrogen deficiency (ND) experiment, six vegetatively propagated tea genotypes were selected: two important cultivars cv. Kolkhida, and cv. Karatum and four mutant forms derived by γ-irradiation of seeds of cv. Kolkhida (#619, #582, #2264, #3823). Among them, cv. Kolkhida and #582 were previously classified as ND-susceptible, while cv. Karatum and #619 – as ND-tolerant.

Sample size: six healthy vegetatively propagated 2-year-old plants were randomly selected for experiments and were sub-cultured to the 4-liter pots, filled with clean river sand; three plants with three replications per treatment.

ND treatment: seven days after subculture, these plants were watered with 50 % of following nutrient solution (pH 5.0–5.1): 0.5 mM $Ca(H_2PO_4)_2$, 3 mM NH_4NO_3, 0.5 mM $CaCl_2$, 1.0 mM K_2SO_4, 46 μM H_3BO_3, 0.6 mM $MgSO_4$, 9 μM $MnSO_4$, 2 μM $CuSO_4$, 9 μM $ZnSO_4$, 2.6 μM Na_2MoO_4 and 30 μM Fe-EDTA. After fourteen days, the experimental plants were watered with 500 ml of 100 % nutrient solution (ND-treatment) or 3 mM NH_4NO_3 (control treatment) each two days [19,20]. During the whole experiment, the plants were maintained at the open-roof greenhouse under the sheding with following conditions: the temperature +24 ± 4 °C, the light intensity of 3000 ± 200 lux, substrate water content of 70 ± 10 %.

Sampling and measurements: the leaf samplings and measurements of indices were conducted after two months of ND. Mature leaves 3rd-4th from the top were sampled to evaluate leaf N-content. Kjeldahl-method was used, including digestion (samples were heated in the presence of sulphuric acid), distillation of the solution and convertation of the ammonium salt to ammonia by addition of sodium hydroxide with the following trapping of the distilled vapours in HCl-water solution. Finally, the amount of ammonia or the amount of nitrogen present in the sample was determined by back titration with neutralization of HCl by NaOH solution [21]. Three biological replicates represented by three different plants per treatment were used for leaf N-measurement.

Five mature leaves per plant were used for measurement of the spectral reflectance (350–1100 nm) by Ci-710s Miniature Leaf Spectrometer (CID Bio-Science, USA). The measurements were conducted at 11 a.m.–3 p.m. The mid part of each leaf (adaxial side beside from the main vehicle) were used for measurements. Totally, 31 VIs were calculated automatically (Supplementary file 1).

Three biological replicates (three different plants) with five technical replicates (five leaves per plant) per treatment were used for reflectance measurement.

2.2. Cold experiment

2.2.1. Plant material and sample size

Two separate plots of 4-year-old F1-offsprings were used for the experiments. These offsprings were derived from the controlled hybridization of freezing-tolerant (A2019) and freezing-susceptible (cv. Kolkhida) parents. First plot (43.5910283° N,39.8292223° E) consisted of 215 plants (109 cold-susceptible and 106 cold-tolerant) and was placed. Second plot (43.5699787° N; 39.7500677° E) consisted of 75 plants (36 – cold-tolerant and 39 cold-susceptible). The degree of freezing tolerance was visually evaluated during 2021–2024 based on: 1. The degree of leaf damage; 2. The vigor of spring vegetation; 3. The degree of damage of new tips (Supplementary file 2, Fig. 1A). Based on these traits, all plants were divided into 5 groups: T1 – most tolerant (n = 66), T2 – tolerant (n = 76), S4 – susceptible (n = 107), S5 – most susceptible (n = 37), S3 – others (excluded from the analysis). These experimental.

Cold treatment: the experimental plants were grown open air in 4-L containers filled with the mix of brown acid forest soil: acid peat = 2: 1, (Fig. 1B). The registration of temperature and air humidity was performed using professional logger EClerk-M-RHT (Relsib, Russia) with verification. The device was placed near the experimental plots to register the temperature dynamics during the studied period (2023–2024) (Fig. 1C).

Sampling and measurements: the following treatments were included in cold-experiment.

1. Control treatment (July 31, 2023) – ten days before this day the minimum air temperature was 19 °C, maximum of 28 °C, with mean value of 23 °C.
2. Cold treatment (December 01, 2023) – ten days before this day the minimum air temperature was of 2 °C, maximum – of 18 °C with a mean value of 10 °C.
3. Freezing treatment (December 28, 2023) – five days before this the minimum air temperature was minus 0.5 °C, maximum of 12 °C, and a mean value was 6 °C; totally 1 h of minus 0.5 °C and 47 h of 0.0–0.4 °C was detected before the freezing-treatment measurements.
4. Recovery treatment (March 19, 2024) – ten days before this day the minimum air temperature was 1 °C, maximum of 21 °C, and a mean value was 8 °C.

Five mature leaves per plant were used for measurement of the spectral reflectance (350–1100 nm) by Ci-710s Miniature Leaf Spectrometer (CID Bio-Science, USA). The measurements were conducted at 11 a.m.–3 p.m. The mid part of each leaf (adaxial side beside from the main vehicle) were used. Totally, 31 VIs were calculated automatically (Supplementary file 1). The number of biological replicates (separate plants) per treatment was 66 (for T1 group), 76 (for T2 group), 107 (for S4 group), 37 (for S5 group). Five technical replicates (five leaves per plant) were used for reflectance measurement.

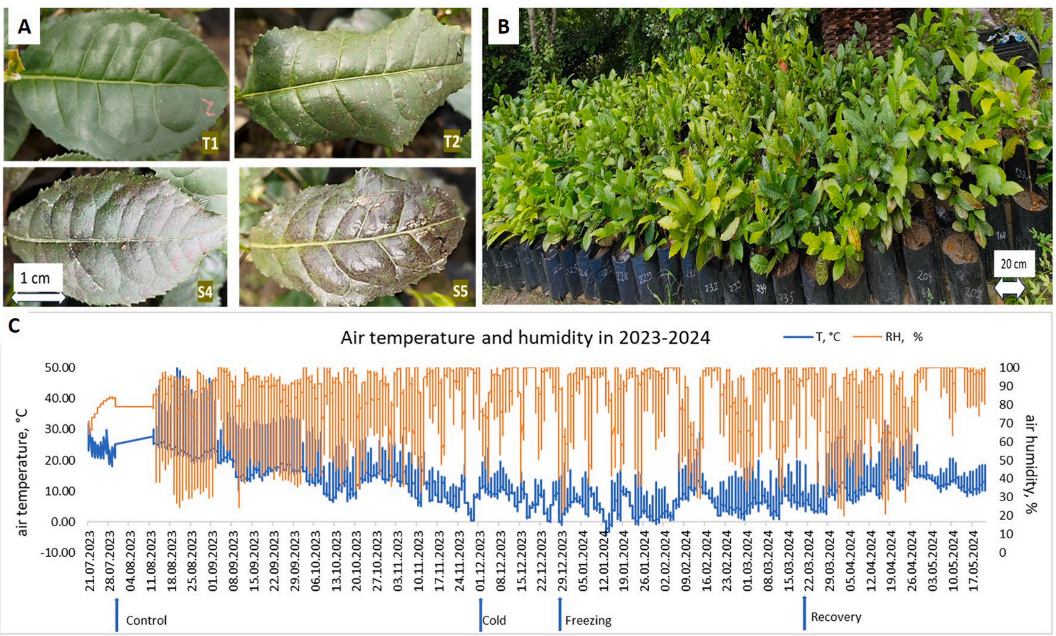

Fig. 1. A – typical tea leaves after the winter frost damage. T1 – most tolerant, T2 – tolerant, S4 – susceptible, S5 – most susceptible; B–F1- offsprings under control conditions (July 31, 2023); C – temperature dynamics in cold experiment.

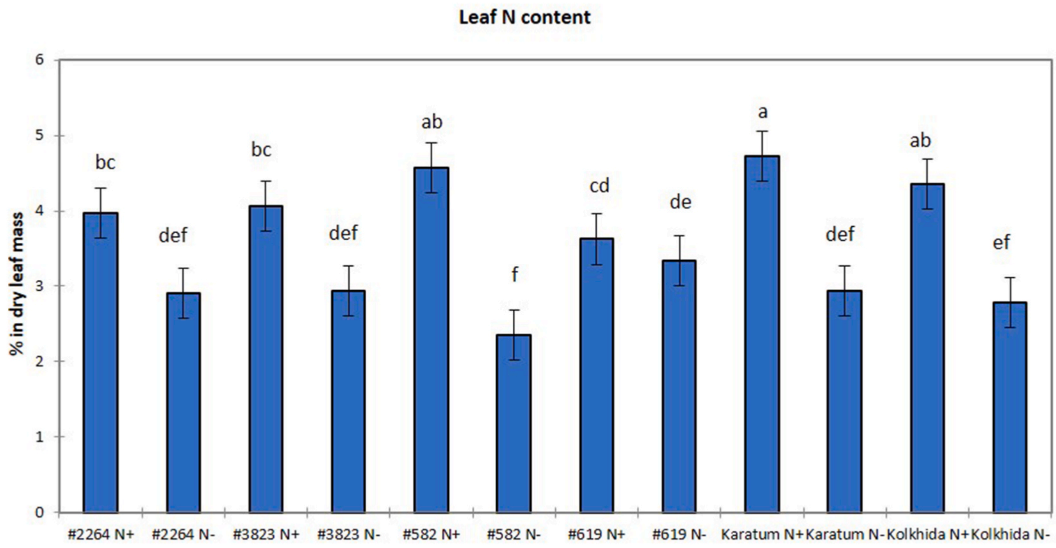

Fig. 2. Effect of the 2-month nitrogen deficiency on leaf nitrogen content in six tea genotypes. Different lowercase letters indicate significance of differences at $p < 0.0001$ according to Tukey' range test. Bars represent standard errors. More details can be found in Supplementary file 3.

2.3. Data analysis

Statistical analyses of data were carried out using XLSTAT software (free trial version) (https://www.xlstat.com/). The multiple comparisons in one-way ANOVA with Fisher's and Tukey's tests were applied to determine significant differences among the respective treatments. Additionally, Pearson (n) PCA and the hierarchical clustering were performed to evaluate the associations among the variables and observations.

3. Results

3.1. Nitrogen deficiency experiment

In control conditions (N+), three genotypes (cv. Karatum, cv. Kolkhida and #582) displayed higher N-content of 4.4–4.7 % as compared to the other genotypes (#2264, #3823 and #619) – 3.6–4.1 % (Fig. 2). ND resulted in significant decrease of leaf N-content in all genotypes, except for #619. The greatest decrease in leaf N content by about 40–50 % was observed in cv. Kolkhida, cv. Karatum and #582. Unexpected result was achieved for cv. Karatum, which was earlier classified as ND-tolerant. Despite this fact, four genotypes were selected for the further VIs assessments: cv. Kolkhida, cv. Karatum, #582 and #619.

Totally, 11 of 31 VIs with the determination coefficients (R^2) of $\geq$0.5 were selected as informative to distinguish ND tolerant tea plants from ND-susceptible ones (Table 1). Under ND-conditions, higher values of ZMI, VREI1, RENDVI, GM1 and GM2 were observed in ND-tolerant genotype #619 as compared to ND-susceptible cv. Kolkhida and #582 (Fig. 3, Supplementary files 1 (abbreviations) and 3 (raw data)). In addition, lower values of Ctr2, MDATT and TCARI were revealed in ND-tolerant genotype #619 as compared to susceptible cv. Kolkhida and #582 (Fig. 3, Supplementary file 3). For example, about 22 % and 10 % RENDVI-decrease, 11 % and 5 % VREI1-decrease, 18 % and 9 % ZMI-decrease were observed in #582 and #619, respectively, under ND. However, most of the studied VIs displayed not significant difference between tolerant and susceptible genotypes. Moreover, the following indices were not efficient to display differences among treatments CRI1, CRI2, CTR1, FRI, G, Lic1, Lic2, MCARI, MRESRI, NPCI, PRI, PSRI, SRPI, WBI, ARI1, ARI2. Finally, the leaf N-content positively correlated with the following indices ZMI, VREI1 and RENDVI, while negatively correlated with CTR2, TCARI, VREI2,3, and MDATT (Table 2).

Table 1
Multiple comparisons' statistics of vegetation indices for evaluation of nitrogen deficiency response in tea plants. More details can be found in Supplementary file 3.

	ZMI	VREI3	VREI2	VREI1	TCARI	MDATT	RENDVI	GM2	GM1	Ctr2
R^2	0.610	0.639	0.641	0.617	0.609	0.574	0.602	0.547	0.592	0.565
F	6.822	7.717	7.807	7.028	6.806	6.149	6.603	5.266	6.323	5.660
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.000	<0.0001	<0.0001	<0.0001	<0.0001

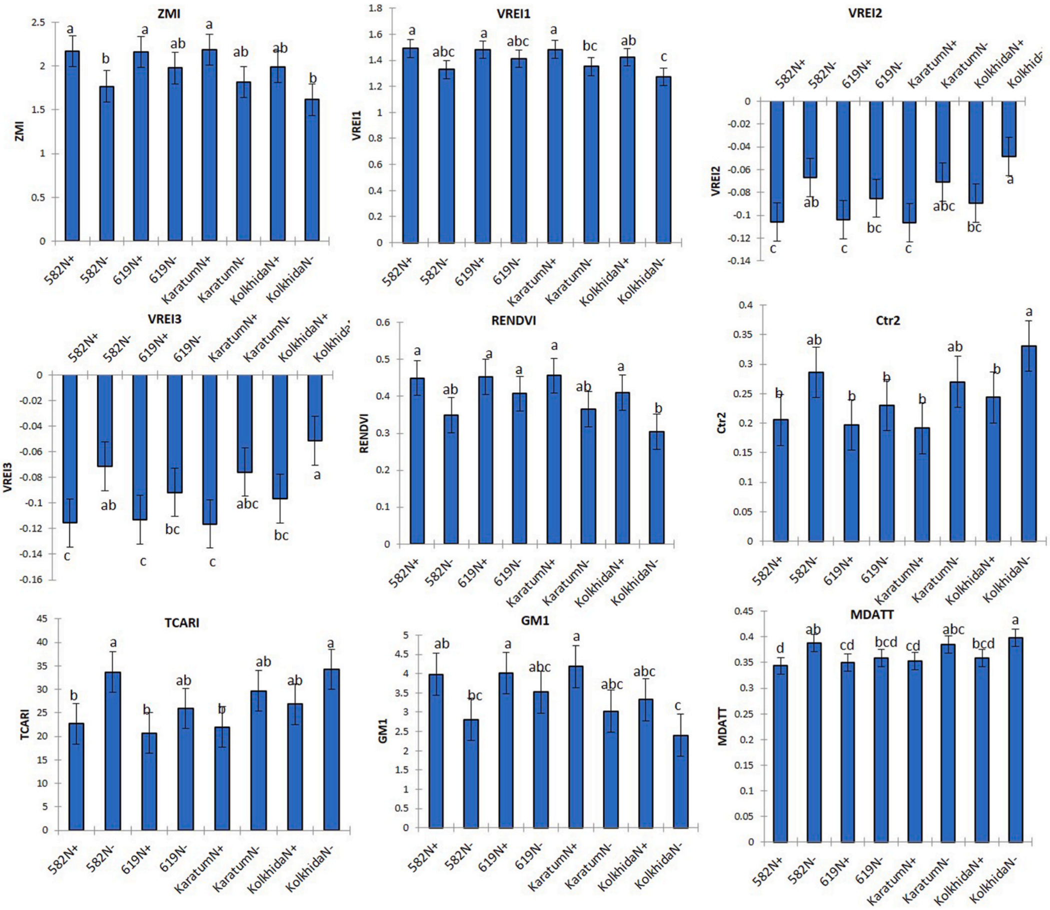

Fig. 3. Mean values of the selected VIs for phenotyping of ND-response of tea plant. Different lowercase letters indicate statistically significant differences between tolerant and susceptible tea genotypes at P value < 0.0001 according to Tukey' range test. Bars represent standard errors. More details and statistics can be found in Supplementary file 3.

Table 2
Pearson's (n) correlations among the studied variables in the nitrogen deficiency experiment (n = 24; p < 0.05). Red shadows indicate positive correlations, blue shadows – negative correlations.

Variables	N content	ZMI	VREI1	SIPI	RENDVI	CNDVI	GM1	Ctr2	TCARI	VREI3	VREI2	MDATT
N content	**1**	**0.619**	**0.626**	**0.379**	**0.605**	**0.605**	**0.611**	**-0.567**	**-0.629**	**-0.633**	**-0.635**	**-0.633**
VREI1	**0.626**	**0.994**	**1**	**0.730**	**0.992**	**0.992**	**0.944**	**-0.961**	**-0.911**	**-0.993**	**-0.993**	**-0.901**
ZMI	**0.619**	**1**	**0.994**	**0.759**	**0.993**	**0.993**	**0.960**	**-0.965**	**-0.907**	**-0.990**	**-0.990**	**-0.884**
GM1	**0.611**	**0.960**	**0.944**	**0.815**	**0.956**	**0.956**	**1**	**-0.941**	**-0.942**	**-0.939**	**-0.938**	**-0.809**
RENDVI	**0.605**	**0.993**	**0.992**	**0.782**	**1**	**1.000**	**0.956**	**-0.985**	**-0.926**	**-0.980**	**-0.981**	**-0.883**
CNDVI	**0.605**	**0.993**	**0.992**	**0.782**	**1.000**	**1**	**0.956**	**-0.985**	**-0.926**	**-0.980**	**-0.981**	**-0.883**
SIPI	**0.379**	**0.759**	**0.730**	**1**	**0.782**	**0.782**	**0.815**	**-0.860**	**-0.737**	**-0.697**	**-0.693**	**-0.506**
Ctr2	**-0.567**	**-0.965**	**-0.961**	**-0.860**	**-0.985**	**-0.985**	**-0.941**	**1**	**0.914**	**0.939**	**0.939**	**0.820**
TCARI	**-0.629**	**-0.907**	**-0.911**	**-0.737**	**-0.926**	**-0.926**	**-0.942**	**0.914**	**1**	**0.896**	**0.898**	**0.827**
MDATT	**-0.633**	**-0.884**	**-0.901**	**-0.506**	**-0.883**	**-0.883**	**-0.809**	**0.820**	**0.827**	**0.902**	**0.907**	**1**
VREI3	**-0.633**	**-0.990**	**-0.993**	**-0.697**	**-0.980**	**-0.980**	**-0.939**	**0.939**	**0.896**	**1**	**1.000**	**0.902**
VREI2	**-0.635**	**-0.990**	**-0.993**	**-0.693**	**-0.981**	**-0.981**	**-0.938**	**0.939**	**0.898**	**1.000**	**1**	**0.907**

Values in bold are different from 0 with a significance level alpha=0.05

Table 3
Multiple comparisons' statistics of vegetation indices for cold-experiment in tea plant (n = 215). Raw data can be found in Supplementary file 2.

Statistics	ZMI	WBI	VREI1	TVI	TCARI	SRPI	SIPI	RENDVI	PSRI	NPCI	MRESRI	MDATT	MCARI1	Lic2	GM2	Ctr1	CNDVI
Control																	
R2	0.010	0.069	0.009	0.074	0.020	0.051	0.006	0.008	0.006	0.057	0.009	0.009	0.074	0.019	0.010	0.016	0.008
F	0.553	3.900	0.459	4.152	1.072	2.806	0.340	0.442	0.303	3.173	0.488	0.452	4.149	1.037	0.535	0.826	0.442
Pr > F	0.697	0.004	0.766	0.003	0.371	0.027	0.851	0.778	0.876	0.015	0.745	0.771	0.003	0.389	0.710	0.510	0.778
Cold																	
R2	0.146	0.681	0.124	0.584	0.315	0.109	0.205	0.101	0.264	0.128	0.445	0.347	0.572	0.739	0.024	0.260	0.101
F	8.684	108.561	7.196	71.386	23.315	6.223	13.123	5.674	18.221	7.459	40.658	26.989	67.827	143.904	1.274	17.850	5.674
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.000	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.281	<0.0001	0.000
Freezing																	
R2	0.155	0.488	0.194	0.119	0.044	0.419	0.244	0.131	0.031	0.321	0.152	0.525	0.166	0.488	0.199	0.647	0.131
F	9.350	48.619	12.277	6.914	2.364	36.775	16.462	7.658	1.643	24.135	9.157	56.458	10.183	48.535	12.666	93.529	7.658
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	0.054	<0.0001	<0.0001	<0.0001	0.165	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Recovery																	
R2	0.193	0.205	0.218	0.048	0.072	0.059	0.025	0.168	0.060	0.056	0.019	0.086	0.036	0.249	0.156	0.287	0.168
F	8.169	8.813	9.541	1.721	2.640	2.165	0.873	6.923	2.188	2.031	0.660	3.206	1.285	11.336	6.327	13.765	6.923
Pr > F	<0.0001	<0.0001	<0.0001	0.149	0.036	0.076	0.482	<0.0001	0.074	0.093	0.621	0.015	0.279	<0.0001	0.000	<0.0001	<0.0001

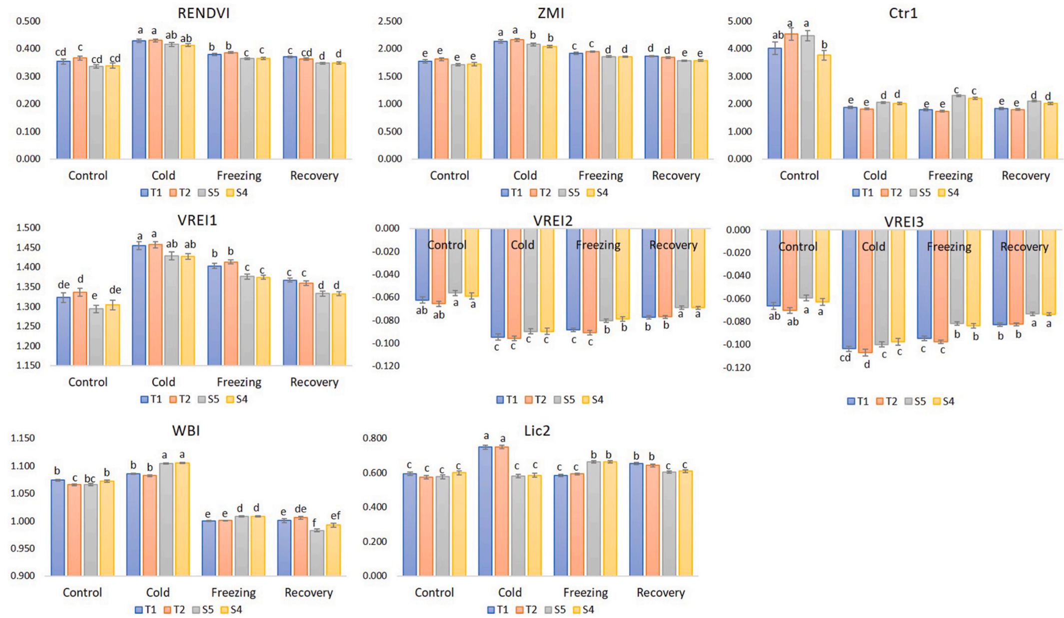

Fig. 4. Mean values of the selected VIs for phenotyping of cold- and freezing-responses in tea plant. Different lowercase letters indicate statistically significant differences between tolerant and susceptible tea genotypes at P value < 0.0001 according to the Tukey range test. Bars represent standard errors. Raw data and more statistics can be found in Supplementary file 2.

3.2. Cold experiments

Generally, low R^2 values were observed in cold experiment. The lowest R^2 of less than 0.1 was observed in control treatment, indicating no significant differences between tolerant and susceptible groups. The Tuckey' range test showed high standard deviations for the following indices ARI1, ARI2, FRI, PRI, G, CRI1, CRI2. Under cold, freezing and recovery treatments, highest R^2 - values (≥10 %) were observed for ZMI, WBI, VREI, RENDVI, Lic2 and Ctr1 indicating significant differences between tolerant and susceptible

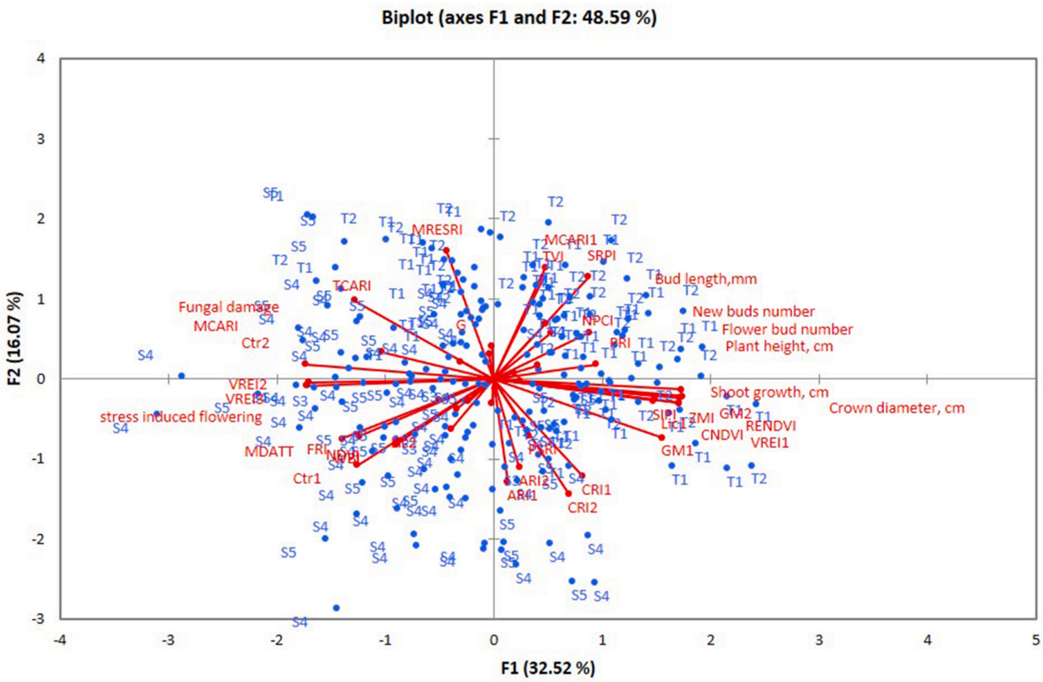

Fig. 5. Principal component analysis of phenotypic traits and vegetation indices in 106 tolerant and 109 susceptible tea genotypes under freezing.

Table 4
Efficient vegetation indices for phenotyping of abiotic-stress response in tea plant (other VIs can be found in Supplementary file 1).

Abbreviation	Index	Equation	Reference
Ctr1	Carter Index 1	(R695/R420)	[22]
Ctr2	Carter Index 2	(R695/R760)	[22]
RENDVI	Red Edge Normalized Difference Vegetation Index	(R750-R705)/(R750+R705)	[23]
VREI1 (VOG1)	Vogelmann Red Edge Index 1	(R740/R720)	[24]
VREI2 (VOG2)	Vogelmann Red Edge Index 2	(R734-R747)/(R715+R726)	[24]
VREI3 (VOG3)	Vogelmann Red Edge Index 3	(R734-R747)/(R715+R720)	[24]
ZMI	Zarco-Tejada & Miller Index	(R750/R710)	[25]

genotypes at $P < 0.0001$ (Table 3). Thus, we further analyzed the significance of these differences between the tolerant and susceptible plants in four treatments (control, cold, freezing, drought).

During each cold, freezing and recovery experiments, tolerant plants were characterized by higher ZMI, VREI1 and RENDVI and lower VREI2,3, WBI, and CTR1 (Fig. 4). As soon as the treatments were independent and statistics models confirmed the significance of the differences, these indices can be proposed as most reliable to identify cold-tolerant plants. Under freezing stress, tolerant tea plants showed the following values of these VIs: ZMI $\geq$1.90, VREI1 $\geq$ 1.40, RENDVI $\geq$0.38, WBI $\leq$0.98, CTR1 $\leq$ 1.74. Besides, susceptible tea plants showed ZMI $\leq$1.85, VREI1 $\leq$ 1.36, RENDVI $\leq$0.36, WBI $\geq$1.00, CTR1 $\geq$ 2.20 (Fig. 4, Supplementary file 2). Lic2 displayed contrasting results: the higher values were observed under cold and recovery, however lower values were detected under freezing in tolerant plants as compared to susceptible ones. Generally, cold treatment displayed greatest values of ZMI, WBI, VREI and RENDVI as compared to control, freezing and recovery treatments. Moreover, control treatment showed lower ZMI, VREI1 and RENDVI and higher CTR1, as compared to cold and freezing treatments. In addition, no significant VIs-differences were observed between tolerant and susceptible groups in control treatment.

We constructed PCA biplot, representing the associated characteristics and relationships of variables (phenotypic traits) and observations (plant groups) (Fig. 5). In control conditions, no clear separation of the tolerant and susceptible accessions was observed, while two PCs showed about 52 % cumulative variation (data are not illustrated here, raw data can be found in Supplementary file 2). Under freezing, first two PCs showed about 49 % cumulative variation and clear separation of the tolerant and susceptible tea plants was observed. Most of the tolerant accessions were distributed on the positive sides of PC1 and PC2, while susceptible ones – on the negative sides of PC1 and PC2. PCA confirmed the results of multiple comparisons' test: vectors of ZMI, VREI1 and RENDVI were placed on the positive side of PC1 with a high loading and associated with tolerant genotypes. In addition, shoot growth activity were positively correlated with these indices. In contrast, stress-induced flowering was related to the cold-susceptible plants and was positively correlated with VREI2, VREI3 and CTR indices, positioned on the negative side of PC1. Based on the results of all experiments, several efficient VIs were revealled for thenotyping of abiotic stress response in tea plant (see Table 4).

4. Discussion

Early non-destructive detection of stress effect is crucial for efficient breeding strategies and germplasm characterization. In this study we aimed to reveal VIs for phenotyping of abiotic stress (cold, freezing, nitrogen deficiency) response in tea plant. Among 31 studied VIs, only few were efficient for distinguishing of tolerant and susceptible tea accessions, namely ZMI, VREI1,2,3, RENDVI and CTR. Most of these indices (except CTR) are calculated based on reflectance area of 705–760 nm, indicating this range as promising for stress prediction and germplasm characterization of tea plant. This result corresponds with some other studies. For example, in tomato ND resulted in increased reflectance, mostly in the wavelength between 775 –850 nm and 910–960 nm [26]. In maize, drought resulted in decreased reflectance in green, red, and NIR regions [27]. Zhao et al. [28] reported that blue or NIR reflectance measurements (R405/R715 and R1075/R735) were linearly correlated with leaf N and chlorophyll contents. Other researchers also observed strong correlation between crop reflectance around 705 nm, 730 nm and 930 nm and chlorophyll content [2,6,8,26,29]. According to the results, increased reflectance in 705–760 nm in tea plant correlated positively with leaf N-content and proposed as the marker of stressed vegetation.

Most of the other studied VIs are calculated based on lower wavelength and were ineffective for tea phenotyping. This corresponds with another study, reported that only ZMI, VREI (VOG 1, 2, 3), RENDVI, and GM2 were efficient among 23 VIs for phenotyping of arctic plant species [30].

Among the efficient indices, ZMI, VREI1 and RENDVI showed higher values in stress-tolerant tea plants. Moreover, we observed positive correlations of these VIs with leaf N-content and shoot growth activity. ZMI was initially proposed as indicator of total chlorophyll [25], while VREI and RENDVI – as indicators of water status of plants [31]. VREI1 (VOG1) (R740/R720) sensitive to the combined effects of foliage chlorophyll concentration, canopy leaf area, and water content [24]. RENDVI (R750-R705)/(R750+R705) differs from NDVI by using red edge bands, instead of main absorption and reflectance peaks [23]. According to the other studies, strong positive correlations were observed between RENDVI and leaf water content in apple [32], potato [33], mint [4] and pear [34] which is consistent with results obtained in this study. In addition, VREI and RENDVI were showed to be less influenced by differences in background than NDVI [35]. Thus, we suggest that these indices can be efficient for remote sensing of tea field plantations.

Among the efficient VIs, Carter indices (CTR1 (R695/R420) and CTR2 (R695/R760)) displayed higher values in susceptible tea plants. These indices are also known as Pigment indices 1 and 2 and proposed as indicators of stress: as chlorophyll degrades, the values of these indices increase [22]. Interestingly, CTR1 was more efficient for cold experiment, while CTR2 – for ND experiment. We suggest that it can be due to the different plant materials used in these experiments. According to the recent studies, CTR1 and CTR2 were efficient for phenotyping of tree species susceptible to Phytophthora under drought stress [36]. Also, these indices were efficient to derive the chlorophyll content of winter wheat under stripe rust stress [37] and to detect the nutritional status of maize [38]. According to our results, stress induced flowering of susceptible plants corresponded with VREI2, VREI3 and CTR (Fig. 3). Moreover, negative correlation was observed between these three indices and leaf N-content (Table 2). Stress induced flowering is a wide-spread phenomenon in various crops [39]. In tea plant, abiotic stresses are known to induce flowering [40]. Particularly, we earlier observed that heat and drought can induce early and abundant flowering of tea plants in last decade of September. Extremely high temperature (near to 40 °C) observed in last decade of August 2023 (Fig. 1C) induced early flowering and induced fungal disease mostly in cold-susceptible plants (Supplementary file 2).

This study displays some surprising results which need to be discussed. Firstly, in cold experiment, lower values of ZMI, VREI1 and RENDVI and higher CTR1 were observed in control as compared to cold and freezing treatments. Besides, control treatment showed no significant differences between tolerant and susceptible plants. It is suggested that leaves were not fully matured, thus were not as green in July (control treatment) as compared to December (cold, freezing treatment), thus the reflectance in July was generally higher than in December.

Secondly, low determination coefficients (R^2) were observed in cold experiment indicating weak relationships between variables and observations. This can probably be explained by big number of plants, included in each group; these plants are seedlings thus having heterogenous genetic background which can increase the error of experiment. However, multiple comparisons statistics were efficient to evaluate the differences between variables (VIs) and observations (genotypes) in each treatment. During each cold, freezing and recovery experiments, tolerant plants were characterized by higher ZMI, VREI1 and RENDVI and lower VREI2,3, WBI, and CTR1. As soon as the treatments were independent and statistics models confirmed the significance of the differences, these indices can be proposed as reliable to identify cold-tolerant tea plants.

Thirdly, WBI (Water Band Index R900/R970) was greater in cold-susceptible plants as compared to the cold-tolerant ones (Fig. 4). WBI is sensitive to changes in canopy water content, as the water content of vegetation canopies increases the strength of absorption around 970 increases related to that of 900 [41]. A significant decrease in the magnitude of the whole NIR reflectance of stressed plants was observed only when the plant was close to wilting [41]. This can be the reason why this index was not efficient in ND experiment. According to the recent study, WBI decreased directly after water stress initiation in monocotyledonous plants (wheat), while in case of dicotyledonous plants (peanut) with double leaf water concentration due to leaf structure capacity, WBI started to decrease when leaf water concentration reached 60 % [42].

Finally, many indices were not efficient for phenotyping of tea plant (for example simple ratio pigment index (SRPI), normalized difference pigment index (NDPI), structure intensive pigment index (SIPI), plant senescence reflectance index (PSRI), Anthocyanin Reflectance Index (ARI), carotenoids reflectance index (CRI), Flavanols Reflectance Index (FRI), photochemical reflectance index (PRI), Modified Chlorophyll Absorption Ratio Index (MCARI), Modified Red Edge Simple Ratio Index (MRESRI), Greenness Index (G), Normalized Pigment Chlorophyll Index (NPCI), Simple Ratio Pigment Index (SRPI)). This is not consistent with several studies which found these indices as efficient [35,43–46]. Thus, obtained results confirm that validation of each VI is necessary for particular crop in different environmental conditions.

5. Conclusion

To conclude, in this study the efficient vegetation indices for phenotyping of abiotic stress response in tea plant were revealed. The following indices were able to distinguish tolerant tea genotypes from susceptible ones ZMI, VREI1,2,3, RENDVI, CTR1 and CTR2. In addition, reflectance area at 705–760 nm were established as promising for stress prediction and germplasm characterization of tea plant. Positive correlations of ZMI, VREI1 and RENDVI with leaf N-content and shoot growth activity were observed. CTR, VREI2 and VREI3 negatively correlated with leaf N-content. These results will be useful for tea germplasm management, for genomic studies and for breeding research aimed at abiotic stress tolerance.

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Data availability

Data are available as Supplementary file 1. All vegetation indices; Supplementary file 2. Cold experiment - raw data and statistics; Supplementary file 3. Nitrogen experiment - raw data and statistics.

CRediT authorship contribution statement

Lidiia Samarina: Writing – original draft, Visualization, Software, Methodology, Investigation, Conceptualization. **Lyudmila**

Malyukova: Writing – review & editing, Validation, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Natalia Koninskaya:** Investigation. **Valentina Malyarovskaya:** Resources, Project administration. **Alexey Ryndin:** Resources, Project administration. **Wei Tong:** Investigation, Formal analysis, Data curation. **Enhua Xia:** Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Elena Khlestkina:** Writing – review & editing, Validation, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35522.

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