



Phenotyping Wild Potatoes for Photosynthesis Associated Traits Under Heat Stress

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Abstract

Heat stress reduces potato productivity by restricting photosynthesis, assimilation production, and sink partitioning. Potato wild relatives (Solanum sect. Petota, Solanaceae) possess abiotic stress resistance characteristics. However, we must assess physiological parameters like gas exchange, chlorophyll index, and fluorescence to determine whether wild potato genotypes can boost crop yield under adverse conditions. We utilized a factorial experimental design to find substantial trait-based genotype differences. The mixed-model technique ranked the genotypes according to their performance in terms of predicted true genotypic values. For potato breeding, we used PCA and cluster analysis on genotypic values to identify critical features and heat-stress-tolerant genotypes. Ranking by best linear unbiased prediction (BLUP) values and heat comprehensive evaluation values for assessed characteristics indicated that predominantly S. chacoense genotypes performed well. Some of the most important physiological characteristics for investigating heat resistant germplasm genetic diversity are net photosynthetic rates, transpiration rates, stomatal conductance, intracellular/ambient CO₂, water usage efficiency, photosystem II operational efficiency, photochemical quenching, and dry matter content. We were able to find genotypes (BGB083, BGB102, BGB103, BGB109, BGB113, BGB444, BGB451, BGB467, and BGB472) that have a mix of these traits and are better at keeping up their photosynthetic performance, water use efficiency, and chlorophyll content. They also have better photoprotective mechanisms that work better when they are under heat stress. Heat stress is most likely to affect BGB008, BGB096, and BGB107. These characteristics are highly valuable for breeding heat-tolerant potato cultivars that can sustain growth, yield, and tuber quality under the increasing threat of heat stress.

Resumen

El agobio por calor reduce la productividad de la papa al restringir la fotosíntesis, la producción de asimilación y la partición de la demanda. Parientes silvestres de la papa (*Solanum* sect. *Petota*, Solanaceae) poseen características de resistencia al

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agobio abiótico. Sin embargo, debemos evaluar parámetros fisiológicos como el intercambio de gases, el índice de clorofila y la fluorescencia para determinar si los genotipos de papa silvestre pueden aumentar el rendimiento del cultivo en condiciones adversas. Utilizamos un diseño experimental factorial para encontrar diferencias genotípicas sustanciales basadas en rasgos. La técnica de modelo mixto clasificó los genotipos de acuerdo con su rendimiento en términos de valores genotípicos verdaderos predichos. Para el mejoramiento de la papa, utilizamos PCA y análisis de conglomerados en valores genotípicos para identificar características críticas y genotipos tolerantes al agobio térmico. La clasificación por los mejores valores de predicción lineal no sesgada (BLUP) y los valores de evaluación integral del calor para las características evaluadas indicó que los genotipos predominantemente de S. chacoense tuvieron un buen desempeño. Algunas de las características fisiológicas más importantes para investigar la diversidad genética del germoplasma resistente al calor son las tasas fotosintéticas netas, las tasas de transpiración, la conductancia estomática, el CO₂ intracelular/ambiente, la eficiencia en el uso del agua, la eficiencia operativa del fotosistema II, el enfriamiento fotoquímico y el contenido de materia seca. Pudimos encontrar genotipos (BGB083, BGB102, BGB103, BGB109, BGB113, BGB444, BGB451, BGB467 y BGB472) que tienen una mezcla de estos rasgos y son mejores para mantener su rendimiento fotosintético, eficiencia en el uso del agua y contenido de clorofila. También tienen mejores mecanismos fotoprotectores que funcionan mejor cuando están bajo agobio por calor. Es más probable que el agobio por calor afecte a BGB008, BGB096 y BGB107. Estas características son muy valiosas para el mejoramiento de variedades de papa tolerantes al calor que pueden mantener el crecimiento, el rendimiento y la calidad del tubérculo bajo la creciente amenaza del agobio por calor.

Keywords Solanum chacoense · Solanum commersonii · Solanum sect. Petota · Solanum tuberosum · Gas exchange · Chlorophyll fluorescence · Genotypic values

Introduction

The potato has seven cultivated species and about 228 wild species (Hawkes 1990). The genus Solanum L. groups them together into Sect. Petota, which is further classified as tuber and non-tuber-bearing species. with the same basic chromosome number (x = 12), ranging from diploid to hexaploid (Hawkes 1990). Wild potatoes are a significant genetic resource for potato breeding because they are utilized and capable of providing resistance genes for environmental and biological challenges that are lacking in commercially grown varieties. Wild potatoes are indigenous to broad portions of South America, from central Argentina and Chile to parts of North America and are found throughout an exceptionally wide range of heights, from sea level to approximately 5,000 m (Correll 1963; Jackson et al. 1978). Three wild potato species are geographically distributed in Brazil: S. commersonii, S. malmeanum, and S. chacoense. These species are distributed in southern Brazil, Uruguay, Argentina, and southeastern Brazil (Hawkes and Hjerting 1969). Given their diverse range of habitats, wild potato species are likely to exhibit varying degrees of genetically determined heat tolerance.

Understanding how climate change affects potato yield is critical for food security. Global climate change-induced extreme heat significantly threatens long-term crop production by diminishing plant performance and output (Dahal et al. 2019; Chourasia et al. 2023). Reports predict that climate change will decrease global potato yields by 18–32% (Hijmans 2003). The impact of heat stress, directly or indirectly related to physiological processes, is responsible for these significant losses in potato yield. It is crucial to use appropriate methods to evaluate the genetic diversity in both inherent and acquired tolerance, as thermotolerance is a polygenic trait (Smillie et al. 1983; Haverkort and Verhagen 2008). We can anticipate varying genetic degrees of stress tolerance in wild potato species, which inhabit a wide variety of settings (Smillie et al. 1983; Tiwari et al. 2022). Breeding programs can utilize several wild potato species that can withstand high temperatures, such as S. kurtzianum, S. sogarandinum, S. chacoense, S. stoloniferum, S. demissum, and S. berthaultii, to create heat-tolerant lines (Guedes et al. 2019). The selection of superior phenotypes, or parents, is the most important phase in crop improvement breeding schemes. According to Rykaczewska (2015), the impacts of heat stress are more detrimental during the early growth phases. However, by selecting the superior genotypes over the base population, the recurrent method of selection can increase the frequency of desirable alleles.

Furthermore, because wild potato species have a significantly larger range of climate adaptation, they may have more potential to cope with supraoptimal temperatures. Physiological phenotyping techniques like gas exchange, chlorophyll fluorescence, and measurement of morphological traits, along with mixed model approaches, can help find wild potato relatives that can be used as new sources of heat tolerance for breeding better potato varieties. Thus, the aim of this study was to identify the potential wild potato ideotype for heat stress, which likely will maintain enough photosynthesis, be effective at utilizing water and fixing more carbon per unit of available water and be able to create a source to sink under heat stress. As a result, under adverse environmental conditions, biomass and tuber production characteristics will increase.

Materials and Methods

Experimental Setup and Growth Conditions

The experiment was carried out in growth chambers of the Phenotyping Platform from Embrapa Clima Temperado, in Pelotas, Rio Grande do Sul, Brazil (32°45′ S and 52°30′ W). Phenotyping was performed for 24 accessions (Table 1) of Embrapa Potato Gene Bank which belong to *Solanum commersonii*, wild; *Solanum chacoense*, wild; *Solanum tuberosum*, di-haploid and one commercial cultivar of *Solanum tuberosum* 4x (BRSIPR-BEL) developed by Embrapa.

Uniform tubers from the randomly selected accessions were taken out of cold chambers and placed on phenolic sponges for 20 days. After acclimatization, tubers were transplanted into a 6-liter plastic pot filled with approximately 5 kg of organo-mineral substrate composed of peat and calcitic limestone, added with N (0.04%), P_2O_5 (0.04%), and K_2O (0.05%). The plants were kept in a greenhouse for the next 15 days until they reached the

emergence stage. After that, we transferred them to growth chambers in three replications, ensuring complete randomization. Hereafter, plants were exposed to two temperature gradients: the control temperature (CT) treatment, with a thermal amplitude of 14 to 27 °C, which is the temperature range for optimum growth for potato crops (Struik 2007), and the heat stress (HS) treatment, with an amplitude of 24 to 34 °C (Fig. 1). The photoperiod was 12 h (7:00 to 19:00 h) with a light intensity of 400 µmol $m^{-2}s^{-1}$, approximately. The accessions used remained under these conditions for 60 days, until harvest time.

Physiological Measurements: Gas Exchange and Chlorophyll Fluorescence

Chlorophyll fluorescence (CF) and gas exchange measurements were carried out at 14, 28, and 42 days after stress (DAS) between time period from 8 a.m. to 1 p.m. Prior to the measurements, the plants were irrigated and kept at a temperature of 24 °C in the control condition and 34 °C in the heat stress condition chambers. After a 20-minute adaptation to these conditions, we evaluated the gas exchange on the third leaf from the top of the stem. The net photosynthesis rate (Pn) µmol CO₂ m⁻²s⁻¹, stomatal conductance (Gs) mol H₂O m⁻²s⁻¹, transpiration

Sr. #	Accession #	Genesys #	Species	Origin
1.	BGB003	BRA 00167009-0	S. commersonii Dunal	Wild, Rio Grande do Sul, Brazil
2.	BGB008	BRA 00167014-0	S. commersonii Dunal	Wild, Rio Grande do Sul, Brazil
3.	BGB009	BRA 00167015-7	S. commersonii Dunal	Wild, Rio Grande do Sul, Brazil
4.	BGB027	BRA 00167203-9	S. commersonii Dunal	Wild, Rio Grande do Sul, Brazil
5.	BGB045	BRA 00167397-9	S. commersonii Dunal	Wild, Rio Grande do Sul, Brazil
6.	BGB083	BRA 00167435-7	S. chacoense Bitter	Wild, Santa Catarina, Brazil
7.	BGB086	BRA 00167438-1	S. chacoense Bitter	Wild, Minas Gerais, Brazil
8.	BGB088	BRA 00167440-7	S. tuberosum L. 2x	Di-haploid of cv. Montana
9.	BGB089	BRA 00167441-5	S. tuberosum L. 2x	Di-haploid of cv. Sowa
10.	BGB091	BRA 00167443-1	S. tuberosum L. 2x	Di-haploid of cv. Anchieta
11.	BGB093	BRA 00167445-6	S. tuberosum L. 2x	Di-haploid of unknown origin
12.	BGB096	BRA 00167448-0	S. chacoense Bitter	Wild, Tucumán, Argentina
13.	BGB098	BRA 00167450-6	S. chacoense Bitter	Wild, Unkown origin, South America
14.	BGB101	BRA 00167018-1	S. chacoense Bitter	Wild, Salta, Argentina
15.	BGB102	BRA 00167019-9	S. chacoense Bitter	Wild, San Luis, Argentina
16.	BGB103	BRA 00167020-7	S. chacoense Bitter	Wild, Córdoba, Argentina
17.	BGB107	BRA 00167024-9	S. chacoense Bitter	Wild, Argentina
18.	BGB109	BRA 00167026-4	S. chacoense Bitter	Wild, Argentina
19.	BGB113	BRA 00167031-4	S. chacoense Bitter	Wild, Salta, Argentina
20.	BGB313	BRA 00167251-8	S. tuberosum L. 4x	Commercial cultivar, Brazil
21.	BGB444	BRA 00167395-3	S. chacoense Bitter	Wild, Santa Catarina, Brazil
22.	BGB451	BRA 00183759-0	S. commersonii Dunal	Wild, Rio Grande do Sul, Brazil
23.	BGB467	BRA 00183774-9	S. chacoense Bitter	Wild, Santa Catarina, Brazil
24.	BGB472	BRA 00183779-8	S. chacoense Bitter	Wild, Santa Catarina, Brazil

Table 1A list of potatogermplasm was evaluatedfrom the Potato Genebank atEmbrapa Clima Temperado,Pelotas, RS, Brazil



Fig. 1 The controlled environment growth chamber is used to expose potato plants to a control temperature (CT) range of 14–24 $^{\circ}$ C and heat stress (HS), a temperature range of 24–34 $^{\circ}$ C, throughout the 24 h until harvest in 2021

rate (E) mmol $H_2O m^{-2}s^{-1}$, and intracellular / ambient CO_2 ratio (C_i/C_a) were measured by using the LI-6400XT photosynthesis system (6400-02B; Li-Cor, Lincoln, NE, USA). The CO₂ concentration used in the Li-COR chamber was 400 µmol mol⁻¹, with a photon flux density of 400 μ mol of photons m⁻²s⁻¹ and a relative humidity of 50-65%. We also used the ratios of Pn, E, and Gs to calculate the water-use efficiency (WUE) and intrinsic WUE as suggested by Farquhar and Richards (1984) and Pimentel et al. (1999). We determined the SPAD values of leaves for the chlorophyll index using a portable chlorophyll meter (SPAD 502 Plus, Minolta, Spectrum Technologies Inc., Illinois, USA). We initiated chlorophyll fluorescence after completing gas exchange measurements. Both growth chambers were adapted to the dark for at least 30 min, as suggested by Murchie and Lawson (2013). The leaves were dark adapted so that the reaction centers were completely open (all primary acceptors oxidized), with minimal heat loss. Then, by using an IMAGING-PAM M-Series 500 fluorometer (Walz Heinz GmbH, Effeltrich, Germany), the initial fluorescence (F_0) in the open centers of photosystem II (PSII) was determined by measuring light (less than 30 μ mol m⁻²s⁻¹), while the maximum fluorescence (F_m) in closed centers or in a reduced state of PSII was evaluated after the application of a 0.8-second pulse of saturation light (7000 μ mol m⁻²s⁻¹). From these values, the potential quantum yield of photosystem II (PSII) was calculated, where $Fv/Fm = (F_m - F_o)/F_m$ (Genty et al. 1989). We applied actinic light (red light) to quantify the steady state of chlorophyll fluorescence. Hence, in the light-adapted

state, the maximum fluorescence (F_m ') was analyzed by the application of a saturating pulse, while the initial fluorescence (F_o ') was evaluated by turning off the actinic light for 2 s after the saturation pulse and turning on the far-red light, and the operating efficiency of photosystem II was calculated as $YII = (F'_m - F_s)/F'_m$ (Baker 2008). Likewise, non-photochemical quenching $NPQ = (F_m - F'_m)/F'_m$, photochemical quenching $qP = (F'_m - F'_s)/(F'_m - F_o)$ were calculated according to Genty et al. (1989).

Measurement of Biomass and Yield Components

At the end of the crop cycle, biomass and yield production components were measured in terms of fresh shoot weight (FSW) in grams, dry shoot weight (DSW) in grams, fresh tuber weight (FTW) in grams, and dry matter content (DMC) in %, as suggested by Bashir et al. (2022).

Statistical Analysis

The analysis of variance (ANOVA) using the general linear model was performed in R (R Core Team 2023) with the *easyanova* v9.0 package (Arnhold 2013). Additionally, for physiological traits data, average was calculated for replicated data which measured at 15DAS, 28DAS and 42DAS were used in analysis.

To calculate the Best Linear Unbiased Predicted (BLUP) or genotypic values (μ +g), procedure model No. 54, as suggested by Mendes et al. (2012), was used in SELEGEN software. The following statistical model was adopted for the evaluation of genotypes in the randomized block design, with one observation per plot and in various two treatments or environments:

$$Y = X_r + Z_g + W_i + e,$$

in which "Y" is the data vector, "r" is the vector of the repetition effects (fixed) summed to the overall average, "g" is the genotypic effects vector (random), "i" is the genotype \times environment effect (random), and "e" is the errors or residues vector (random). The capital letters (X, Z, and W) represent the matrix of incidence for the aforementioned vector effects.

The predicted genotypic or BLUP values (μ +g+ge) of used accession for each environment (CT and HS). Ratio across treatments (HS/CT) of BLUP values for each genotype was calculated using following formula: *BLUP_{HS}/BLUP_{CT}*. The calculated ratios were used to make bar graph of predicted genotypic values for all analyzed variables.

The $\mu + g + ge$ refers to the average genotypic value across all environments and includes an average interaction with all the environments evaluated, was used to carry out the principal component analysis (PCA) biplots, clustering, and correlation analysis in R Studio using R packages, Factoextra (Kassambara and Mundt 2020), FactoMineR (Lê et al. 2008), and Corrplot (Wei and Simko 2021). Hierarchical cluster analysis (HCA) uses Ward's method to classify the genotypes into

Table 2 Results from analysis of variance (degree of freedom, DF; means square, MS) for physiological traits (SPAD) chlorophyll index, (pn) net photosynthesis rate, (gs) stomatal conductance, (E) transpiration rate, (C i /C a) intercellular and ambient CO 2 . Ratio, (WUE) water use efficiency, (iWUE) intrinsic water use efficiency, (YII) operating efficiency of PSII, (NPQ) non-photochemical quenching, (qP) photochemical quenching, (Fv/Fm) potential quantum yield of PSII and morphological variables (FSW) fresh shoot weight, (DSW)

different clusters based on the squared Euclidean distance calculated by the Ward's method of transformed z-score values grouped the evaluated genotypes into clusters based on the similarities in the performance of evaluated traits.

Data was merged and cleaned by using the R package Tidyverse (Wickham et al. 2019b). Mean comparison graphs for variables, DAS, and genotypic value figures were prepared using ggplot2 (Wickham. et al. 2019a).

Tolerance Analysis

The membership function value method is used to comprehensively evaluate potato characteristics associated with heat tolerance. The main principal component (PC) was used to calculate the heat comprehensive evaluation value (HCEV). HCEV was then used to rank the used potato accession by their phenotypic response as calculated by the formulas proposed by (Zhang et al. 2024).

Results

Genotypic Responses of the Potato Germplasm

The analysis of variance (ANOVA) results of the mean squared difference for all examined traits are presented in

dry shoot weight, (FTW) fresh tuber weight, (DMC) dry matter content, considering 24 potato germplasm genotypes (G) and two temperature treatments (T) as sources of variation and their interaction. *DF* degree of freedom, $G \times T$ interaction effect of genotype and treatment, $G \times DAS$ interaction effect of genotypes and day after stress. ^{NS,*,***}, and ^{****} are significant at P > 0.05, $P \le 0.05$, $P \le 0.01$, and $P \le 0.001$, respectively

Source of variance	Genotype (G)	Treatment (T)	$G \times T$	Days after stress (DAS)	T × DAS	
DF	23	1	23	2	2	
SPAD	3.56***	9.04***	0.36 ^{NS}	11.15***	0.47^{NS}	
Pn	2.67^{***}	2.24^{*}	0.44 ^{NS}	5.32***	6.06***	
Gs	1.65***	22.40^{***}	0.51 ^{NS}	3.90***	9.46***	
E	1.84^{***}	0.08 ^{NS}	0.84^{*}	3.00**	15.92***	
C _i /C _a	0.53 ^{NS}	70.11***	0.50 ^{NS}	1.08^*	8.28^{***}	
WUE	0.81^{*}	19.78***	1.27^{***}	0.92^{NS}	15.29***	
iWUE	0.99^{**}	50.49***	0.42^{NS}	0.91 ^{NS}	7.60^{***}	
YII	2.23****	1.31 ^{NS}	0.43 ^{NS}	9.12***	0.66^{NS}	
NPQ	1.74^{**}	7.59**	0.66 ^{NS}	1.70 ^{NS}	4.42**	
qP	2.24^{***}	3.63*	0.52^{NS}	7.98***	0.34^{NS}	
Fv/Fm	1.74^{***}	23.56***	0.45^{NS}	12.41***	0.82 ^{NS}	
FSW	3.75***	7.37***	0.73**			
DSW	3.53***	6.23***	0.67^{*}			
FTW	4.06^{***}	8.10^{***}	0.54^{***}			
DMC	3.84***	3.10***	0.32 ^{NS}			



<Fig. 2 Mean comparison of measured traits after 14 days after stress (DAS), 28DAS and 42DAS among 24 genotypes of potato (*Solanum*, Solanaceae) under CT- control temperature and HS- heat stress conditions. Letters indicate significant differences based on Tukey's HSD test ($\alpha \le 0.05$)

Table 2, which shows a significant difference among the genotypes and treatment factors for most of the traits. The interaction effect $(G \times T)$ for E, WUE, FSW, DSW, and FTW was significant. For DAS, we observed significant differences in physiological traits, except for WUE, iWUE and NPQ. The interaction effect of treatment × DAS was significant for all traits except for SPAD, YII, qP, and Fv/ Fm (Table 2). The mean comparison of all studied variables related to physiological under both CT and HS conditions for the time points (DAS) was shown in Fig. 2a-k, and the mean comparison of morphological traits for both treatments was presented in Fig. 2. Overall, the chlorophyll index (SPAD), WUE, iWUE, and qP were significantly higher under HS by 8.99%, 55.33%, 256.75%, and 11.57%, while Gs, C_i/C_a, Fv/Fm, and all the morphological traits (FSW, DSW, FTW, and DMC) showed a significant reduction under HS conditions (Fig. 2). However, there was no significant difference observed for the performance of Pn, E, and YII (Fig. 2).

A significant reduction due to HS was observed for the physiological traits Gs, C_i/C_a , NPQ, and Fv/Fm ratios by 45.37%, 36.18, 8.26, and 2.63, and for all morphological traits FSW, DSW, FTW, and DMC by 20.11%, 11.53%, 50.92%, and 50.97% as compared to CT conditions (Fig. 2). Only SPAD significantly and gradually decreased through time when observed on 14, 28, and 42 DAS. Pn, E, YII, and qP showed little or no reduction after 14 and 28 DAS, but a significant reduction was measured after 42 DAS. C_i/C_a , Fv/Fm ratios were higher at 14 DAS, and at later points, ratios were reduced but remained constant (Fig. 2). C_i/C_a , NPQ, and iWUE did not show any change in responses to time/ plant development (Fig. 2).

The predicted genotypic $(\mu + g + ge)$ responses of all used potato germplasm for physiological and morphological traits were presented in S. Figs. 1, 2, and 3; Supp. T1; Fig. 3. All the genotypes were ranked according to their BLUP value for both treatments and mean lines showed an average of all germplasm in the respective treatment. The bars above the mean lines showed a higher performance than the average of the total germplasm used. Subsequently, we ranked genotypes with better performance of the respective trait under the given conditions first, and those with the worst response to the trait last. The ratio across treatment (HS/ CT) above 1 showed a higher predicted genotypic value trait performance under HS as compared to predicted genotypic values under CT condition of the respective genotype. Moreover, ratio closer to 1 indicates a low difference in the performance of genotypes for a trait between both conditions

(Supp. T1). Trait ratios across treatment (HS/CT) were used to investigate the magnitude of treatment effect on each trait. Differences in yield components including FTW and DMC capture the contribution physiological characteristics observed throughout plant development and can be considered a measurement of productivity penalty under heat stress. Genotypes BGB008, BGB045 from *S. commersonii*, and BGB467 (*S. chacoense*) had the smallest difference in FTW and DMC penalty under HS, whereas *S. chacoense* accessions (BGB083, BGB102, BGB444, BGB451) had the greatest difference (Fig. 3; S. Table 1).

Potato genotypes evaluated for net photosynthesis rate, water use efficiency, intrinsic WUE (S. Fig. 1), operating efficiency of PSII, photochemical quenching (S. Fig. 2) and chlorophyll index (S. Fig. 3) showed higher predicted genotypic values under HS. In case of stomatal conductance, intracellular/ambient CO_2 ratio (S. Fig. 1), non-photochemical quenching, potential efficiency of PSII (S. Fig. 2), dry shoot weight (S. Fig. 3), dry matter content and fresh tuber weight (Fig. 3), predicted genotypic values of the used accessions were higher under CT conditions as compared to HS. However, 72% of the *S. chacoense* accessions predicted to have higher genotypic values for transpiration rate (S. Fig. 1) under CT as compared to HS (Supp. T1).

Potato accessions were ranked according to the genotypic performance for the SPAD values (S. Fig. 3). Nine genotypes included (BGB027, BGB091, BGB093, BGB101, BGB103, BGB107, BGB109, BGB113, and BGB313) showed a higher value than the total average for the trait under both conditions (S. Fig. 3). These genotypes are classified as better performers for SPAD values. For net photosynthesis rate (S. Fig. 1; Supp. T1) BGB091 (S. tuberosum 2x) was ranked first, followed by the S. commersonii accessions BGB003, BGB009, BGB008, and BGB045, along with the S. chacoense accessions (BGB101, BGB107), and the commercial cultivar BGB313. These accessions were also better performers for the Gs, E, and C_i/C_a than the average performance of these traits (S. Fig. 1; Supp. T1). S. tuberosum 2x accessions (BGB089, BGB091, and BGB093) showed better genotypic values for Gs, E, C_i/C_a, and WUE (S. Fig. 1).

The commercial cultivar BRSIPR-BEL (BGB313) recorded the highest predicted genotypic values for YII and qP (S. Fig. 2) and lowest HS/CT ratio for the traits (Supp. T1). Five genotypes belonging to *S. chacoense*, two accessions of *S. commersonii*, and one *S. tuberosum* 2x accession showed higher BLUP values than the overall mean for NPQ (S. Fig. 2; Supp. T1). Among these accessions, BGB009, BGB101, BGB109, BGB467, and BGB472 were predicted to have higher Fv/Fm genotypic values. However, genotypes BGB003, BGB027, BGB086, BGB093, BGB098, BGB103, and BGB313 were predicted to have lower NPQ genotypic performance but better potential for PSII efficiency (S. Fig. 2; Supp. T1).



Fig.3 Predicted genotypic values $(\mu+g+ge)$ for (**a**) Fresh tuber weight (g); **b** dry matter content (%) of potato genotypes of each treatment, and error bar represents confidence interval (CI), horizon-

Long-term heat stress considerably affects the vegetative and tuber traits of the studied potato germplasm. The FSW, DSW, FTW, and DMC were significantly reduced due to HS (Fig. 2). BGB045, BGB098, BGB101, BGB103, BGB109, BGB444, BGB451, and BGB467 accessions were observed to have greater than average values for FSW and DSW than the average (S. Fig. 3). The highest FTW predicted genotypic values were observed for BGB113 under both conditions, while all three accessions of S. tuberosum 2x (BGB088, BGB091, BGB093), S. chacoense (BGB102, BGB103, BGB109, BGB444, BGB451, BGB472), and BGB313 (S. tuberosum 4x) also performed higher than the general mean of the genotypic values under HS conditions (Fig. 3). All the S. chacoense genotypes except BGB467 were predicted to have a higher genotypic value for DMC than the average of the trait (Fig. 3). Moreover, FSW BLUP values for genotypes BGB091, BGB113, BGB093 and BGB088 were higher under HS (S. Fig. 3) and BGB083, BGB451 have higher FTW genotypic values under HS, while all the other accessions predicted to have higher values under CT conditions (Fig. 3).

Principal component analysis (PCA) based on genotype level BLUP estimates of photochemistry, gas exchange parameters, and morphological traits was used to evaluate the variation present among the used potato germplasms. The PCA identified the four principal components marked as PC1 to PC4 as influential, with Eigen values > 1.0 accounting for 82.61% of cumulative contribution rate (Table 3). The first PC explained the highest variation present among the genotypes by the studied traits under both conditions and accounted for 45.36% of the cumulative percentage of variation explained. Traits such as Pn, Gs, E, C_i/C_a, WUE, iWUE, YII, qP, and DMC were the most influential traits contributing to the variance with the first PC. These traits form part of the second main component, accounting for 15.84% of the total variance and 61.20% of the cumulative total variance



tal dashed lines represent the grand mean of the trait under respective treatment and bars are arranged in a descending order with highest BLUP value over the average of both treatments

observed. The chlorophyll index (SPAD), WUE, iWUE, YII, NPQ, qP, Fv/Fm, and FTW have the highest values for the eigenvector contribution (Table 3). PC3 was mainly related to Fv/Fm, FSW, DSW, and FTW and PC4 was mainly related to non-photochemical quenching (NPQ).

The biplot had four main axes (Fig. 4), with the upper right axis having a positive impact on PC1 and PC2, and the genotypes located have been demonstrated by different shapes according to their corresponding species. Blue arrows represent physiological traits, and red arrows represent morphological traits. The distance, or degree of angle, between the two arrows represents the correlation present among the traits. Cluster analysis of 24 potato accessions were classified into three groups based on inertia gain (Fig. 4). The characteristics of each cluster were also summarized (Fig. 5). Cluster-I is comprised of genotypes including BGB083, BGB102, BGB103, BGB109, BGB113, BGB444, BGB451, BGB467, and BGB472 (Fig. 4). All the genotypes in this group belong to S. chacoense, and they account for 37.5% of the total germplasm analyzed. Cluster-I is summarized by the lowest genotypic values for Pn, Gs, E, C_i/C_a ; however, there is a positive association of the WUE, iWUE, FSW, DSW, and DMC traits with the respective cluster (Fig. 5). Cluster-II contains only three accessions (BGB008, BGB096, and BGB107). NPQ and Fv/Fm ratios are the only two traits significantly associated with this cluster. Therefore, the group's accessions are higher in NPQ genotypic values and lower in Fv/Fm ratios than the overall mean of the respective traits.

Cluster-III contains the highest number of accessions, 50% of the total potato genotypes evaluated (BGB003, BGB009, BGB027, BGB045, BGB086, BGB088, BGB089, BGB091, BGB093, BGB098, BGB101, and BGB313). This group includes all accessions of *S. tuberosum* 2x commercial cultivar (BGB313), four accessions of *S. commersonii*, and three accessions of *S. chacoense*. The accessions belonging

Table 3Loading matrix,Eigenvalues, and the variancecontribution rate of theprincipal component calculatedfrom the average BLUP valuesfrom evaluated environments(CT and HS), estimated from a15-variable model for 24 potatogenotypes

Index	Principal	component			Weight of principal component					
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4		
SPAD	0.14	0.76	-0.38	0.34	0.05	0.49	-0.28	0.32		
Pn	0.87	0.08	0.03	0.38	0.33	0.05	0.02	0.36		
Gs	0.88	-0.02	0.03	0.33	0.34	-0.02	0.02	0.31		
Е	0.96	0.04	0.06	0.23	0.37	0.03	0.05	0.22		
C_i/C_a	0.89	0.10	0.13	-0.05	0.34	0.07	0.10	-0.04		
WUE	-0.75	0.36	-0.13	0.24	-0.29	0.23	-0.10	0.23		
iWUE	-0.81	0.33	-0.04	0.07	-0.31	0.22	-0.03	0.07		
YII	0.81	0.48	0.02	-0.09	0.31	0.31	0.02	-0.08		
NPQ	-0.39	-0.51	0.05	0.60	-0.15	-0.33	0.03	0.57		
qP	0.80	0.36	-0.05	0.02	0.31	0.23	-0.04	0.02		
Fv/Fm	0.24	0.49	0.51	-0.40	0.09	0.32	0.38	-0.38		
FSW	-0.44	0.29	0.78	0.22	-0.17	0.19	0.57	0.21		
DSW	-0.51	0.38	0.67	0.18	-0.20	0.25	0.50	0.17		
FTW	-0.34	0.48	-0.51	-0.11	-0.13	0.31	-0.38	-0.10		
DMC	-0.53	0.48	-0.25	0.12	-0.20	0.31	-0.18	0.12		
Eigen	6.81	2.38	1.83	1.12						
CR %	45.36	15.84	12.22	7.48						
CRR %	45.36	61.20	73.42	80.90						
Weight	0.56	0.20	0.15	0.09						

PC1, PC2, PC3, PC4, four top principal components; CR contribution rate, CCR cumulative contribution rate

to this cluster showed totally contrary results as compared to Cluster-I. The genotypes showed higher average genotypic values for Pn, Gs, E, C_i/C_a , YII, and qP but lower genotypic values for WUE, iWUE, NPQ, and DMC (Fig. 5).

Since a single morphological or physiological index could not serve as a reliable indicator of heat tolerance in crops, heat comprehensive evaluation value (HCEV) based on multiple indexes were used for evaluating the germplasm (Fig. 6). HCEV was calculated based on the chlorophyll index (SPAD), net photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (E), water use efficiency (WUE), intrinsic water use efficiency (iWUE), intracellular/ambient CO_2 ratio (C_i/C_a), operating efficiency of PSII (YII), non-photochemical quenching (NPQ), photochemical quenching (qP), potential quantum efficiency of PSII (Fv/ Fm), fresh shoot weight (FSW), dry shoot weight (DSW), fresh tuber weight (FTW), and dry matter content (DMC). The HCEV value of the 24 potato germplasm resources ranged from 0.32 to 0.59 (Supp. T2; Fig. 6). Wild potato accession BGB109, BGB113, BGB102, BGB472, BGB467 belong to S. chacoense exhibited a significantly higher HCEV and were ranked top 5. This value exhibited a weak association with heat tolerance as measured by the HS/CT ratio of productivity traits (Fig. 6).

The correlation plot (Fig. 7) demonstrates that gas exchange traits (Pn, E, Gs, C_i/C_a) found highly positive and significant associations with each other, but these traits had

significant negative associations with WUE and iWUE. The accessions that showed better performance for YII were also found to be better for the qP and demonstrated by the significant positive correlation present among them. Photochemistry traits YII and qP were also found to be significantly correlated with gas exchange traits (Fig. 7). The Fv/Fm ratio was positively associated only with YII. DMC was found to be negatively correlated with Pn, Gs, E, and C_i/C_a . However, DSW and DMC positively correlated with iWUE, WUE, and FTW (Fig. 7). Among the photochemistry traits NPQ was the only trait that was found to be negatively correlated with all gas exchange traits but significantly with qP and YII (Fig. 7).

Discussion

Effective germplasm screening for heat tolerance, particularly under heat stress conditions, using various morphological and physiological attributes, is an effective way of selecting materials for advanced breeding programs. The study's results reveal that HS induced increases in leaf chlorophyll content (SPAD values) are real and repeatable due to consistent increase at three different measurement times 14, 28, and 42 DAS (Fig. 2). These responses propose that potato plants increase their leaf chlorophyll content as an acclimation response to HS. The SPAD index potentially



Fig. 4 Dispersion and clustering of 24 potato (*Solanum*, Solanaceae) genotypes from the Embrapa Potato Genebank by principal component analysis were evaluated for the chlorophyll index (SPAD), net photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (E), water use efficiency (WUE), intrinsic water use efficiency

(iWUE), intracellular/ambient CO_2 ratio (Ci/Ca), operating efficiency of PSII (YII), non-photochemical quenching (NPQ), photochemical quenching (qP), potential quantum efficiency of PSII (Fv/Fm), fresh shoot weight (FSW), dry shoot weight (DSW), fresh tuber weight (FTW), and dry matter content (DMC)

exhibit some predictive ability for potato productivity under heat-stress conditions. Research on wild potato genotypes reveals a non-significant, positive correlation between SPAD values and tuber yield (Fig. 7), suggesting that potato breeding programs may be able to use this trait as a proxy marker for heat tolerance (Demirel et al. 2017). Studies on other Solanaceae species have also used SPAD values to check the amount of chlorophyll and how well they can response to heat (Nemeskéri and Helyes 2019; Bhattarai et al. 2021; Haque et al. 2021). SPAD is one of the main parameters typically measured, along with other gas exchange parameters (Demirel et al. 2017; Tang et al. 2018) to screen potato germplasm. Under HS, the plants could have gained an upward shift in the optimum temperature, providing thermostability to PSII, which most likely prevented the degradation of chlorophyll molecules (Havaux 1993). Therefore, this leads to increased photosynthetic activity under HS (Hancock et al. 2014). In our study, Pn increased by 13% on average under HS (Fig. 2). Similar results were reported for the heat-resistant potato genotypes such as Desirée and Norchip (Hancock et al. 2014; Aien et al. 2017) and indicate that heat-tolerant genotypes can sustain higher photosynthesis levels. Also, as the HS period lasted longer, potato germplasm was observed to maintain the Pn. This was shown by the fact that photosynthesis activity was significantly higher after 28 DAS under HS, however after 42 days of growth, there was no difference of Pn observed under HS and CT conditions (Fig. 2). Although, Pn observed under both conditions after 42 DAS was lower than the initial stages (14 DAS, 28 DAS). This suggests that wild germplasm has the capability to keep the photosynthesis machinery stable (Jha et al. 2014; Janni et al. 2020) by potentially through mechanisms of upregulation of heat shock proteins (HSPs), and increase in the production



Fig. 5 Phenotypic clusters. Box plots show medians, 25 and 75 percentiles, and whiskers indicate 1.5 times the interquartile range of the variables assessed within each cluster. The x-axis shows the cluster performance of the respective traits on the y-axis. Each point rep-

resents the genotype's best linear, unbiased predictor. The asterisk above the error bar indicates the significant association of the following trait with the respective cluster by p value: $* \le 0.05$, $** \le 0.01$, $*** \le 0.001$



Fig. 6 Heat comprehensive evaluation values (HCEV) of 24 potato (*Solanum*, Solanaceae) genotypes from the Embrapa Potato Genebank calculated by the morphological and physiological index and ranked from highest to lowest HCEV



Fig. 7 The correlation between the average genotypic values in the various environments capitalizes on an average interaction with all evaluated environments of the studied physiological and morphological traits. The intensity of the color of the square increases with an increase in the correlation. Dark red denotes a high negative correlation, whereas dark blue denotes a high positive correlation. Cells with an asterisk denote the level of significant correlation between the variables. The full form of abbreviations used is given as follows:

of antioxidants that ultimately preventing photosynthetic damage (Jha et al. 2014; Hu et al. 2020) and productivity under elevated temperatures (Hastilestari et al. 2018). This is a desirable trait for breeding heat-tolerant varieties, as it indicates the ability to sustain yield potential under HS conditions (Gautam et al. 2024). Superior photosynthetic traits found in wild potato germplasm can be potentially used to make potato cultivars that can better handle heat stress

chlorophyll index (SPAD), net photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (E), water use efficiency (WUE), intrinsic water use efficiency (iWUE), intracellular/ambient CO2 ratio (C_i/C_a), operating efficiency of PSII (YII), non-photochemical quenching (NPQ), photochemical quenching (qP), potential quantum efficiency of PSII (Fv/Fm), fresh shoot weight (FSW), dry shoot weight (DSW), fresh tuber weight (FTW), and dry matter content (DMC)

(Wang-Pruski and Schofield 2012; Guedes et al. 2019; Lal et al. 2022).

Plants adjust their stomatal conductance and transpiration rates to regulate leaf temperature and avoid excessive heat buildup. This helps maintain photosynthesis by keeping the leaves cool (Giorno et al. 2013; Hu et al. 2020). In our study, potato genotypes close stomata significantly during heat stress (Fig. 2; S. Fig. 1). Stomatal closure during HS follows a stress avoidance strategy (Jensen 1981). The strong association between Pn and Gs suggests that it can balance CO₂ intake while minimizing water loss (Fig. 7). All of the accessions evaluated significantly decreased Gs (S. Fig. 1), according to predicted genotypic values. However, we observed that accessions (BGB003, BGB008, BGB009, BGB027, BGB086, BGB088, BGB089, BGB091, BGB093, BGB098, BGB101, BGB109, and BGB313) had higher predicted genotypic values for transpiration under the HS condition (Fig. 2; S. Fig. 1). This indicates that these accessions have the potential to avoid HS by increasing water loss through passive processes other than stomatal transpiration. Hence, the higher E and lower Gs indicate a more efficient regulation of water use and better adaptation to heat stress. The reduced stomatal conductance helps to minimize stomatal water loss, which is crucial for maintaining plant water status and avoiding heat-induced wilting and yield reductions (Bai et al. 2023). The remaining genotypes conserve water by reducing stomatal activity (Gs), which leads to efficient water management due to lower E (Sadok et al. 2021). Many studies have found comparable findings in various crops (Berry and Bjorkman 1980; Jolliet and Bailey 1992; Aien et al. 2017; Gu et al. 2017; Sadok et al. 2021; Aldubai et al. 2022). These physiological responses are often associated with enhanced thermotolerance in plants (Chaudhary et al. 2020). By understanding the genetic basis and mechanisms behind these traits, breeders can identify and select the most promising wild potato accessions to incorporate heat tolerance into their breeding programs (Dempewolf et al. 2017; Janni et al. 2020).

Intracellular to ambient CO_2 (C_i/C_a) ratio under HS is a complicated and growing topic of study that might also boost potato yields and tolerance to rising temperatures (Lee et al. 2020; Ahmadi-Lahijani et al. 2021). Research indicates that plants capable of maintaining a C_i/C_a ratio of 0.6 or higher tend to exhibit greater heat tolerance (Tan et al. 2017). Such ratios indicate effective stomatal regulation, allowing plants to optimize water use while maintaining adequate carbon uptake for photosynthesis (Ehleringer and Cerling 1995). This screening trial showed that more than 50% of germplasm (S. Fig. 1) had genotypic values higher than 0.6, as indicated by Bunce (2005). The lower C_i/C_a values under HS (Fig. 2) suggest improved stomatal regulation, allowing the plant to conserve water while maintaining CO_2 uptake for photosynthesis (Jha et al. 2014).

Overall, all accessions in our study showed higher genotypic values for WUE and iWUE under HS (S. Fig. 1). The observations suggest that the wild genotypes adopting a optimizing mechanism (Borba et al. 2017; Priya et al. 2018) and reduce stomatal conductance to conserve water, leading to decreased transpiration rates (Bashir 2022), coupled with maintained or enhanced carbon assimilation, results in improved WUE and iWUE (Fig. 2; S. Fig. 1) under HS. Jha et al. (2014) also reported this key adaptation in legumes which is desirable for keeping productivity high under HS. Therefore, our study suggests that phenotyped germplasm potentially has novel phenotypic or genetic combinations that confer superior WUE and iWUE under HS (Bethke et al. 2019).

Photoinhibition, as a negative mechanism for protecting PSII, limits photosynthesis (Guidi et al. 2019). Using photoinhibition indicators, this study estimated potato germplasm diversity to survive heat stress. The Fv/Fm ratio is a crucial indicator of potato plant stress (Krause and Weis 1991; Baker 2008). The *S. commersonii* accessions BGB003, BGB009, BGB027, and BGB045 had a higher genotypic value under CT than the Fv/Fm ratio average under HS. So, did the *S. tuberosum* 2x accession BGB093 and the seven *S. chacoense* accessions BGB086, BGB098, BGB101, BGB103, BGB109, BGB467, and BGB472 (S. Fig. 2).

However, other genotypes showed reduced Fv/Fm ratios, indicating photoinhibition (Ogren 1991; Krause and Winter 1996; Bertamini and Nedunchezhian 2003; Santabarbara et al. 2019). All phenotyped germplasm showed predicted Fv/Fm genotyping values > 0.7, as observed by Baker (2008). Fv/Fm ratios depend on the YII (Kitajima and Butler 1975) and photoinactivation due to the PSII reaction center closure (Campbell and Tyystjärvi 2012); hence, accessions with lower YII and qP tend to have lower Fv/Fm ratios (S. Fig. 2). In addition, excess energy, or NPQ under HS, also influences Fv/Fm (Malnoë 2018). We predict that accessions with lower NPQ genotypic values (S. Fig. 2) will have higher Fv/Fm ratios or better stress tolerance.

Potato development is both indeterminate and determinate depending on the cultivar and environment conditions, and HS can redirect photoassimilates from sink tissue to other tissues, which can change tuberization, bulking, and tuber output (Viola et al. 2001; Fleisher et al. 2006). Moreover, dry matter content productivity is associated with the distribution of photoassimilates (Dahal et al. 2019; Koch et al. 2019). But in this study under HS most of the S. tuberosum accessions showed lower FSW and DSW genotypic values (S. Fig. 3) while gained higher FTW than the average of the trait (Fig. 3) and most of the S. chacoense genotypes maintained their higher values under HS for FSW, DSW and DMC. This suggests that they can survive HS and may have ways to allocate the photoassimilte to sink tissue for tuber bulking. A non-significant correlation between these traits (Fig. 7) explains this behavior of not adopting a similar mechanism to cope HS for all the germplasm. Tubers with increased dry matter may respond to stress. Higher dry matter concentration implies storage compound buildup; however, it may reduce tuber size and water content (Appeldoorn et al. 1997). According to genotype responses to FSW, the plant may be investing more in dry matter than the fresh weight of tubers.

Our study reveals a positive correlation between the SPAD index genotypic values and tuber yield, measured in terms of fresh tuber weight, although it is not significant. This suggests that the wild genotypes can tolerate heat stress by increasing their leaf chlorophyll but this trend does not translate to greater tuber yields under elevated temperature. Demirel et al. (2017) also found the same trend in potatoes, and Barutcular et al. (2016) found a correlation between SPAD values and yield in Poaceae family crops. González et al. (2011) found a significant positive correlation between Gs, E, C_i/C_a , Pn, YII, and qP, indicating a close link between these traits under HS conditions. This implies that the same underlying mechanisms, likely related to stomatal regulation and gas exchange, influence all these physiological parameters (González et al. 2011). This information allows us to focus on selecting a combination of these positively correlated traits for program breeding, as improving one trait is likely to lead to improvements in the others. On the other hand, the significant negative correlation between Gs, Pn, E, C_i/C_a , WUE, and iWUE is also informative (Fig. 7). This inverse relationship indicates a tradeoff between maximizing carbon assimilation and water use efficiency under HS conditions. This trade-off indicates that the plants are prioritizing the maintenance of essential physiological processes, such as maximizing carbon assimilation and energy production, over the accumulation of carbohydrates and other storage compounds in the tubers. The fact that NPQ and YII are negatively correlated suggests that when potato plants are under a lot of HS, they need to dissipate energy through NPQ, but this gets in the way of them using light energy efficiently (González et al. 2011; Reddy et al. 2020). This reflects the wild potato germplasm's ability to protect its photosynthetic apparatus and its capacity to maintain high photosynthetic efficiency under adverse conditions. Therefore, genotypes with lower NPQ and higher YII values are likely to be more heat-tolerant, as they can better preserve the efficiency of their photosynthetic machinery under stress. These negative associations also highlight the importance of identifying and selecting potato genotypes that can strike a balance between these traits.

PCA can reduce the number of dimensions without much loss of information based on their similarities, differences and define a limited number of 'principal components' which describe independent variation structures in the data (Shin et al., 2011). The PCA technique has been employed for main economic character evaluation as well as for classification or clustering (Karim et al 2022; Placide et al. 2015). Using genotypic responses of wild germplasm, the PCA explained 81% of the variation in the germplasm that was studied and helped us with genotypes that exhibit similar values of these traits under CT and HS conditions (Table 3). PCA suggests that Pn, Gs, E, C_i/C_a, YII, qP, and DMC are features that capture the majority of independent variation in these germplasm, under the combined CT and HS conditions (Banerjee et al. 2020; Al-Ashkar et al. 2023). It is not logical to assume that only one trait is indicative of the heat tolerance of potato genotypes. The PCA along with cluster analysis (Fig. 4) and in combination with subordinate function (Supp. T2) allows us to group and rank the genotypes using HCEV (Fig. 6). This approach has been frequently applied to evaluate the tolerance of germplasm in many crops (El-Hendawy et al. 2005; Tian et al. 2015; Sun et al. 2016; Al Mahmud et al. 2021; Fan et al. 2022) but is not a direct measurement of heat tolerance in this experiment.

Therefore, by using PCA values, clustering of wild potato germplasm allows us to identify wild potato ideotypes with similar responses. Thus, Cluster-I comprises just S. chacoense accessions (Fig. 4). This group may preserve their average Pn phenotype, it is also shown by the significant differences present between HS/CT ratios of the genotypes present in cluster-I with II and III (Table 4). The closed stomata of Cluster-I genotypes (Fig. 5) made them less sensitive to CO_2 . However, they used water more efficiently, as shown by higher WUE and iWUE values and less water loss (E), which caused C_i/C_a ratios to drop. The genotypes in this group had better heat dissipation (NPQ) ability and PSII operational efficiency, leading to better stress tolerance (Fv/Fm). They also have higher FSW and DSW, and some genotypes in this group may send photoassimilates (like starch and carbohydrates) to sink tissues, which raises FTW and DMC. Perhaps surprisingly, minimal significant differences were seen in the HS/CT ratio of yield productivity

Table 4The statistical significance of the differences between clusters of wild potato accessions obtained from the PCA matrix, considering theHS/CT ratios of the parameters by the Student's t test

Cluster	SPAD	Pn	Gs	Е	C _i /C _a	WUE	iWUE	YII	NPQ	qP	Fv/Fm	FSW	DSW	FTW	DMC
I vs. II	-	*	*	_	_	***	_	_	_	_	_	_	_	_	_
I vs.III	-	**	***	***	***	***	-	***	-	***	-	-	-	**	-
II vs. III	-	-	-	**	-	-	-	***	-	**	-	-	-	-	-
R-squared	0.25	0.42	0.61	0.22	0.61	0.70	0.29	0.21	0.08	0.23	0.04	0.11	0.03	0.19	0.41

*, **, *** significant difference at the 0.05, 0.01 and 0.001 level ($P \le 0.05$; $P \le 0.01$; $P \le 0.001$), respectively

traits (FTW, DMC) across the three groups (Table 4). These results suggest that indeed methods like PCA and HCEV are effective at grouping genotypes by phenotypic similarity, but do not provide a measurement of agronomic productivity under heat stress tolerance. Furthermore, morphological traits did not significantly contribute to elucidating the tolerance levels of wild genotypes about HCEV. It is essential to assess the physiological characteristics for measuring the tolerance levels of wild accessions, as indicated by other potato research (Zhang et al. 2024). HCEV indicates that Gs, Ci/Ca, and WUE may serve as the most effective indicators for assessing genotypic differences because of their significant correlation and magnitude, while Pn and DMC may be considered as other options for examining tolerance levels. Our findings suggest that there are many different ways wild potato genotypes can be optimized to turn light energy into CO₂ fixation when growing in HS under limited abiotic conditions. Future studies are needed to understand the relationship between photosynthetic parameters, physiological characteristics, plant biomass production, and how these traits influence tuber yield, and dry matter content under heat stress. Results from this study may be helpful addressing these outstanding questions in the future through generation of genetic mapping populations that segregate for the traits presented.

Conclusion

Finally, using BLUP genotypic values, we were able to rank the genotypes based on their performance on the specific trait. PCA analysis, on the other hand, employs genotypic values to cluster wild germplasm based on trait ideotype similarity. Based on this classification, we may conclude that genotypes with a balance of these features have a greater ability to maintain photosynthetic activity, water use efficiency, and enhanced photoprotective mechanisms, resulting in higher tuber production under heat stress conditions. Furthermore, the HCEV ranking provides a different way of assessing wild germplasm focused on the balance of measured traits. As a result of these findings, genotypes that ranked first by BLUP values or belong to the first cluster or top six genotypes according to tolerance analysis can be focused on one trait that leads to an improvement in the cultivar's overall heat tolerance ability due to the significant association present with other most influential traits in explaining the variability. This targeted approach can boost the breeding program's efficiency and success in developing climate-resilient potato cultivars. These features are extremely beneficial for developing heat-tolerant potato cultivars that can maintain growth, yield, and tuber quality in the face of rising heat stress.

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Data Availability Data is available upon request.

Declarations

I, Ikram Bashir, the corresponding author, declared on behalf of all authors that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. There has been no involvement that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

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