

Phenotyping wild potatoes (*Solanum* Sect. *Petota*) for photosynthesis associated traits under heat stress.

Ikram Bashir (✉ ikrambashir215@gmail.com)

Faculdade de Agronomia Eliseu Maciel, Programa de Pós-graduação em Agronomia, Universidade Federal de Pelotas, Brasil. <https://orcid.org/0000-0002-0076-4620>

Rodrigo Nicolao

Faculdade de Agronomia Eliseu Maciel, Programa de Pós-graduação em Agronomia, Universidade Federal de Pelotas, Brasil. <https://orcid.org/0000-0002-6542-9583>

Willian Silva Barros

Instituto de Física e Matemática, Departamento de Matemática e Estatística, Universidade Federal de Pelotas, Brasil <https://orcid.org/0000-0003-1057-6006>

Janni André Haerter

Embrapa Clima Temperado <https://orcid.org/0000-0003-4742-9042>

Giovani Greigh de Brito

Embrapa Clima Temperado <https://orcid.org/0000-0003-4268-6197>

Caroline Marques Castro

Embrapa Clima Temperado <https://orcid.org/0000-0003-4605-0014>

Gustavo Heiden

Embrapa Clima Temperado <https://orcid.org/0000-0002-0046-6500>

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Abstract

Heat stress is one of the major factors reducing potato yield by limiting the plant photosynthesis activity, production and partitioning of assimilates to the sink. Potato wild relatives (*Solanum* sect. *Petota*) are a natural reservoir of valuable traits for abiotic stress resistance. However, it is necessary to assess the response of wild potato genotypes based on physiological features such as gas exchange, chlorophyll index and chlorophyll fluorescence that are promising candidates to improve crop photosynthesis under adverse conditions. Multivariate approaches on two factor experimental design were used to select the best performing genotypes for most important traits influencing the photosynthetic activity under control and heat stress treatments. The most important traits to be focused on are net photosynthetic rates, transpiration rates, and stomatal conductance along with effective photochemical yield of PSII, Coefficient of photochemical yield of PSII, electron transport rate and Fv/Fm ratio which are highly associated with heat stress conditions. *Solanum commersonii* accessions (BGB003, BGB009) are more likely to be tolerant to heat stress, followed by *S. tuberosum* 2x (BGB091, BGB093) and *S. chacoense* (BGB101). The assessment of wild accessions for photosynthetic traits under heat stress is a reliable way to discriminate the most promising sources of abiotic stress resistance even though they have unreliable tuber traits for the breeding of the potato crop.

Introduction

Plant regulatory mechanisms have evolved to adapt to various weather changes, such as fluctuating irradiation, limited and excessive precipitation, or low and high temperatures. Despite the inhibition of their photosynthetic processes, these adaptations allow plants to thrive even in rapidly changing conditions (Ulukan, 2008; Zivcak et al., 2017). As a result, the success of crop species and genotypes is determined by the efficiency of the photosynthetic apparatus (Ghotbi-Ravandi et al., 2014; Jurczyk et al., 2015).

The increase in average global temperature is modeled to reach 0.3–4.8°C by the end of the XXI century (IPCC et al., 2021), which directly affects the growth and development of plants by changing photosynthesis and growth period. This scenario will lead agriculture to a challenge, especially in arid regions. Basically, this temperature increase can cause a reduction in the stomatal conductance and rate of photosynthesis (Urban et al., 2017) by warming up mesophyll cell and causing increase in the temperature of the stroma and thylakoid membrane within the chloroplasts (Scafaro et al., 2011). However, Havaux (1992) stated that exposure of potato (*Solanum tuberosum* L., Solanaceae) leaves to heat stress (HS) caused an increase in activity of photosystem II (PSII) in stressed plants due to the quick adaptation of the potato leaves to elevated temperature conditions. This happens because photosynthesis and gas exchange involve several processes related to the conversion and transformation of radiation into the photochemical energy powering CO₂ fixation and the formation of assimilates, which is essential for the plant's survival during heat stress.

Therefore, non-invasive technique such as chlorophyll fluorescence (CF) and infra-red gas analyzer (IRGA) has become a well-established and commonly used to assess the photosynthetic performance of plants by physiologists and eco-physiologists (Maxwell and Johnson, 2000). The CF and IRGA has been used to detect and quantify the damage in photosystem II (PSII) in several crops including potato (Sundbom et al., 1982), wheat (Wu and Bao, 2011; Sharma et al., 2015), rice (Gu et al., 2017) and oats (Rizza et al., 2001) under various abiotic stress conditions. This technique allows rapid and early phenotyping of genotypes to identify existing genetic variation in responses of the stomatal conductance (Gs) and rate of photosynthesis (Pn) (Urban et al., 2017) associated with heat stress tolerance. During heat stress, high temperatures can cause damage photosynthetic components, leading to a decline in chlorophyll fluorescence, which could be observed with decline in the effective photochemical efficiency of photosystem (YII) (Guidi et al., 2019; Muhie, 2022). This decline in photochemical yield suggests that the plant is undergoing stress, and its ability to carry out photosynthesis is being compromised (Allel et al., 2018; Huang et al., 2011), which can be indicated by an important stress indicator (Baker, 2008) called maximum chlorophyll fluorescence (Fv/Fm). Additionally, it has been postulated that non-photochemical quenching (NPQ) plays a key role in determining plant productivity (Demmig-Adams et al., 2014). According to (Hubbart et al., 2018), NPQ may contribute to enhance productivity by decreasing the photoinhibition, but may also reduce the plant yield by overly protective persistence, lowering quantum yield when a plant is submitted to low light unnecessarily (Kromdijk et al., 2016). Several authors have suggested that chlorophyll fluorescence can be more efficiently used as a **suiTable** selection criterion for genotype screening and as an indicator of the effects of climate change on crop species (Sawatraksa et al., 2018; Bezerra-Neto et al., 2022; Grzesiak et al., 2023). The ability of plants to adapt photosynthetic response in changing environments depends on the level of genetic variation within a population. One of the major approaches for improving photosynthetic efficiency of crop species could be through exploitation of natural variation for photosynthesis rates in wild relatives/germplasms (Crop Trust, 2023). Therefore, geographically widely distributed potato genepool comprises more than 100 wild relative species (Spooner et al., 2014) and containing valuable reservoir of allelic genetic diversity for various biotic and abiotic stresses incorporated into potato breeding program over time (Bashir et al., 2021).

Hence, climate change, especially the increase of temperature, requires identifying new sources of heat tolerance and breeding new potato cultivars able to cope with supraoptimal temperatures. Physiological phenotyping approaches such as gas exchange in combination with chlorophyll fluorescence may help to identify and phenotype best performing genotypes, aiming define new plant ideotype to face future climate scenarios. Thus, the aim of this study was to identify the ideal wild potato ideotype for heat stress which likely will achieve maximize photosynthesis, be effective at utilizing available water by accessing more water and fixing more carbon per unit of water taken up, hence will be resulted in maintaining tuberization under conditions of high temperature. However, wild potatoes tuber traits were not focused in this study because high heterosis of bad tuber quality traits such as secondary growth, high levels of glycoalkaloids, difference in tuberization timing are the drawback for the potato breeding program.

Material and methods

Plant material and temperature treatments:

The experiment was carried out in controlled environment, at the Phenotyping Platform from Embrapa Clima Temperado, in Pelotas, Rio Grande do Sul, Brazil (32°45' S and 52°30' W). Tubers of uniform sizes from 21 accessions (**Table 1**) randomly selected from Embrapa Potato Gene Bank were placed on phenolic sponges and, after 20 days of acclimatization, were transplanted to plastic bags filled with approximately 4 kg of organo-mineral substrate. The plants were kept in a greenhouse until 15 days when they reached the emergence stage (Jefferies et al., 1991). Subsequently, they were taken to growth chambers and exposed to two temperature gradients: control temperature (CT) treatment, with a thermal amplitude of 14 to 27°C, which is the temperature range for optimum growth for potato crop (Struik, 2007) and heat stress (HS) treatment, with an amplitude of 24 to 34°C, the same as we applied in our previous study (Bashir et al., 2022). The photoperiod was 12 hours (7:00 to 19:00h) with a light intensity of 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$, approximately (**Fig. 1**). The evaluated plants remained under these conditions for 60 days until the time of harvest. The data were recorded after 15 days after stress (DAS), 28DAS and 42DAS in factorial experimental design.

Chlorophyll fluorescence:

Chlorophyll fluorescence analyses were performed using the IMAGING-PAM M-Series 500 fluorometer (Walz Heinz GmbH, Effeltrich, Germany). Before measurements, the plants were adapted to the dark within each growth chamber for at least 30 minutes, and the temperature was maintained at 24°C for control condition and 34°C for stress condition. For measurements, actinic light of 7000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photon was activated to measure the different states of chlorophyll fluorescence and parameters Fv/Fm, Y(II), NPQ, qP and ETR derived from the equations described in **Table 2**, to which mean values correspond to 281, 336, 396 and 461 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PAR.

Leaf gas exchange:

Measurements were performed by using the LI-6400XT photosynthesis system (6400-02B; Li-Cor, Lincoln, NE, USA) on the third expanded leaf from the top of the stem in a plant of each genotype previously irrigated and adapted for at least 30 minutes at a temperature of 24°C for control condition, and 34°C for stress condition. The CO₂ concentration used in the chamber was 400 $\mu\text{mol mol}^{-1}$ and a photon flux density of 400 $\mu\text{mol of photons m}^{-2}\text{s}^{-1}$, and relative humidity 50–65%. Net photosynthesis rate (Pn) $\mu\text{mol C}_2\text{O m}^{-2}\text{s}^{-1}$, stomatal conductance (Gs) $\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$, transpiration (E) $\mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$, and intracellular and ambient CO₂ assimilation ratios (Ci/Ca) were calculated according to (von Caemmerer and Farquhar, 1981) with the software of the Li6400. Water-use efficiency (WUE) was measured by taking the ratio of photosynthesis rate and transpiration, defined as Pn/E (Farquhar and Richards, 1984) and intrinsic WUE (Pimentel et al., 1999) was proposed, defined as Pn/Gs .

SPAD Chlorophyll Index

Dimensionless chlorophyll content measurements (SPAD 502 Plus, Minolta, Spectrum Technologies Inc., Illinois, USA) were made. Each leaf was measured thrice (on either side of the mid-rib).

Statistical analysis:

Statistical studies were performed in R Studio (Kronthaler and Zöllner, 2021), including two factorial ANOVA, correlation, principal component analysis, and clustering. The cluster score (Nagar et al., 2015) was derived as a weighted linear combination of physiological traits (i.e., summation of weightage multiplied with their respective physiological trait).

Results

Analysis of variance (ANOVA) results for all examined traits is presented in **Table 3**, which shows significant difference among the traits and genotypes under control (CT) and heat stress (HS) conditions. The mean value of different traits such as SPAD chlorophyll index, net photosynthetic rate, transpiration rate, effective photochemical quantum yield of photosystem II, Coefficient of photochemical fluorescence quenching and electron transport rate showed increasing trends, while a decreasing trend by remaining traits under heat stress conditions in this study (**Fig. 1**). The bar graph showed significant differences among the genotypes under control and stress conditions. It represents the mean values with group lettering for the studied traits (**Fig. 1**). The mean values of all traits measured on 14, 28 and 42 days after stress in both control and stress conditions.

Performance of studied germplasm

The data for the SPAD chlorophyll index (SPAD) were recorded under both temperature ranges, CT and HS. Under CT, SPAD values ranged 30.64–46.80 with a mean of 36.11. Under HS, it ranged 31.46–53.75 with a mean of 39.33 (**Fig. 2**). In heat stress condition (HS), *S. chacoense* BGB109 showed the maximum value of SPAD, also it showed the maximum value under control conditions (CT). However, *S. commersonii* BGB009 showed minimum under HS conditions and *S. chacoense* BGB096 showed minimum under CT conditions (Supplementary **Table 1**). The net photosynthesis rate (Pn) of potato germplasm under control condition ranged from 3.99–15.00 with a mean rate of $9.89 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$. In HS conditions, it ranged from 5.41–17.53 with an average of 11.15 (**Fig. 2**). Under control temperature conditions, *S. commersonii* genotype BGB009 showed the highest while *S. chacoense* genotype BGB102 showed the lowest values for net rate of photosynthesis. In stress conditions (HS), *S. tuberosum* 2x genotype BGB091 attains the maximum values and minimum photosynthesis rate genotype BGB083 exhibits the minimum values (Supplementary **Table 1**).

Stomatal conductance increased from 0.10 to 0.75 and showed a mean value of 0.41 in control temperature condition and from 0.03 to 0.66 with mean value of $0.22 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ under stress conditions (HS) as listed in Supplementary **Table 1**. Genotypes BGB107 and BGB109 are the best and

worst performers belonging to *S. chacoense* specie group under control condition (CT) while *S. commersonii* genotypes BGB003 and BGB451 had the highest and lowest stomatal conductance respectively, under HS condition (Supplementary **Table 1**).

The transpiration rate (E) of the given data had mean values of 4.42 and 4.34 mmol H₂O m⁻²s⁻¹ in control and heat stress conditions, respectively. *S. commersonii* genotype BGB003 exhibited the high transpiration rate among all studied genotypes in both CT and HS conditions. The lowest transpiration rate was shown by *S. chacoense* BGB102 and *S. commersonii* BGB451 under control and stress conditions (Supplementary **Table 1**). *S. commersonii* genotypes BGB045 and BGB003, showed the high intracellular and ambient CO₂ assimilation ratio (Ci/Ca) under both applied conditions (**Fig. 2**), while *S. chacoense* BGB109 was negative under stress and *S. tuberosum* 2x BGB093 had low Ci/Ca in control condition, respectively (Supplementary **Table 1**). Observed values for CO₂ assimilation ratio (Ci/Ca) under CT and HS conditions have significant differences. Mean values under control were 0.82 and for stress was 0.43 (Supplementary **Table 1**).

Water use efficiency (WUE) and intrinsic water use efficiency (iWUE) (**Fig. 2**) for the studied genotypes showed mean values 2.33 and 32.30 under CT conditions and 3.59 and 113.91 under HS, respectively. In the case of WUE, 5 genotypes showed higher values in CT as compared to the HS. BGB102, *S. chacoense* and BGB093, *S. tuberosum* 2x showed the low and highest values in CT while BGB045, *S. commersonii* and BGB109, *S. chacoense* under HS conditions. However, *S. commersonii* showed higher WUE, followed by *S. chacoense* and *S. tuberosum* 2x under stress treatments. Furthermore, BGB045 and BGB109 showed the lowest (37.91) and highest (300.54) iWUE under heat stress conditions, respectively. *S. commersonii* genotypes have the highest iWUE values, followed by *S. tuberosum* dihaploid and *S. chacoense* in HS conditions (Supplementary **Table 2**).

The recorded data from chlorophyll fluorescence traits; effective photochemical quantum yield of photosystem II YII in heat stress (HS) ranged 0.10–0.29 with mean value of 0.20 while under control condition mean value was slightly low 0.18 and ranged between 0.13–0.25 (**Fig. 3**). *Solanum tuberosum* 2x genotype BGB093 showed highest value and *S. chacoense* BGB083 had lowest value for Y(II) under normal temperature range. In case of heat stress environment, *S. commersonii* BGB045 had high and *S. chacoense* BGB113 had low effective quantum yield (YII) (Supplementary **Table 2**).

Nonphotochemical quenching (NPQ) under control temperature ranged between 0.48–0.74 with a mean value of 0.59. Under HS conditions, the value ranged from 0.39–0.68 with the mean value of 0.54. BGB096 (*S. chacoense*) had the highest value for NPQ under both CT and HS treatments, while BGB093 (*S. tuberosum* 2x) and BGB045 (*S. commersonii*) had the lowest NPQ in both conditions, respectively (Supplementary **Table 2**). High value for coefficient of photochemical quenching (qP) was exhibited by commercial cultivar BRS-BEL in both studied environments. However, qP under control condition ranged 0.27–0.51 and BGB467 *S. chacoense* has the lowest values and *S. tuberosum* 2x BGB093 the highest one with mean value of 0.37. In heat stress conditions qP ranged from 0.23 for BGB444 *S. chacoense* to 0.52 for BGB003 *S. commersonii* with mean of 0.41 (Supplementary **Table 2**).

The data showed that electron transport rate (ETR) had a mean value of 27.39 and 29.97 in both tested conditions respectively with BEL showing the highest ETR. Under heat stress conditions, *S. commersonii* BGB045 showed the highest rate, and the lowest rate was shown by BGB113 *S. chacoense*. In control temperature range, *S. tuberosum* 2x BGB093 had the highest ETR, while *S. chacoense* BGB083 had the lowest rate among all tested genotypes (Supplementary **Table 2**). The analysis of data showed that changes in maximum photochemical quantum yield of photosystem II (Fv/Fm) between the control and stress conditions were very significant. The Fv/Fm of studied germplasm under normal conditions ranged 0.75–0.78. under control treatment, genotype BGB467 had the highest result but genotype BGB096 had the lowest result, both belongs to *S. chacoense*. The genotype BGB003 had the highest values along with BEL, BGB086 and BGB472, while the lowest values were observed for the genotypes BGB113 and BGB107 of *S. chacoense*. The mean values for the studied genotypes were 0.75 and 0.73 under control and stress treatment (Supplementary **Table 2**).

The mean data presented in (Supplementary **Tables 1 and 2**) showed the average performance of species of which different genotypes were studied under control and stress treatments. In stress condition (HS), *S. tuberosum* 2x species characterized as high SPAD chlorophyll index, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (E), intracellular and ambient CO₂ assimilation ratio (Ci/Ca), effective photochemical quantum yield of PSII (YII), coefficient of photochemical quenching (qP) and high electron transport rates (ETR). *S. chacoense* showed high water use efficiency (WUE), intrinsic water use efficiency (iWUE), non-photochemical quenching (NPQ) along with maximum photochemical quantum yield of photosystem II (Fv/Fm). Under control temperature (CT), *S. commersonii* germplasm observed to be with high Pn, Gs, E and Ci/Ca. *Solanum tuberosum* 2x showed high SPAD index, WUE, iWUE, YII, qP and ETR, while germplasm belonging to *S. chacoense* performs highest with NPQ and Fv/Fm traits.

Correlation among photosynthetic traits

Correlation of 24 genotypes for all studied chlorophyll fluorescence and photosynthesis traits were shown in **Fig. 4**, under control temperature (CT) range and heat stress (HS) condition. Net photosynthesis rate (Pn) had positive and highly significant association with the stomatal conductance (Gs), transpiration rate (E), intracellular and ambient CO₂ assimilation ratio (Ci/Ca), effective photochemical quantum yield of photosynthesis II (YII), coefficient of photochemical quenching (qP) and electron transport rates (ETR) but negative significant association with water use efficiency (WUE) and intrinsic water use efficiency (iWUE) under the heat stress condition. However, under HS, nonphotochemical quenching (NPQ) was found significant negative correlation with Y(II) and ETR while positive and significant correlation with WUE, iWUE. In stress conditions, the maximum photochemical quantum yield of photosystem II (Fv/Fm) showed a medium significant association with YII and ETR, whereas in control conditions, Fv/Fm showed no significant association with any traits. Stomatal conductance (Gs) and transpiration rate (Tr) had a strong positive correlation, as did CO₂ assimilation ratios, effective photochemical yield, photochemical coefficient, and electron transport rates (ETR). Water use efficiency (WUE) and intrinsic water use

efficiency (iWUE) have positive significant correlation among them, whereas negative significant correlation with qP, Y(II), ETR, Pn, E, Gs and Ci/Ca.

In CT, net photosynthesis rate (Pn) showed medium to high significant positive associations with electron transport rates (ETR), photochemical yield (YII), coefficient of photochemical quenching (qP), stomatal conductance (Gs), and transpiration rates (E), and water use efficiency. Under heat stress conditions, SPAD chlorophyll values showed no significant correlation with any of the studied traits, whereas under control conditions, it showed a low significant correlation with YII, qP, and ETR shown in **Fig. 4A & B**.

Genetic diversity of physiological traits

The principal component analysis (PCA) based on Chlorophyll fluorescence and photosynthesis traits identified the six principal components (PC) under both CT and HS as significant with Eigen values > 1.0 accounted for 89.75%, respectively (**Fig. 5**). The six three principal components (PC) 46.60%, 16.48%, 9.98%, 7.24%, 4.98% and 4.46% contribute to the total variance, respectively. The first and second PC explained by the high positive correlation of the studied traits. Traits such as Y(II), ETR, qP, Pn, Gs, WUE, E measured under CT conditions and E, Gs, Pn, Ci/Ca, Y(II), ETR and qP under HS conditions showed highest correlation with first principal component. In second principal component (PC2) iWUE for CT and SPAD for both conditions (CT & HS) have the high positive correlation (**Table 4**).

The first PC was negatively correlated by Ci/Ca, NPQ, iWUE in CT and NPQ, WUE, iWUE under HS. Under CT conditions, PC2 showed negative values for NPQ, Pn, E, Gs and Ci/Ca for both CT and HS. A biplot was generated between first two PC in both conditions (CT & HS). The biplot had four main axes, with the upper right axis having a positive impact on PC1 and PC2, and the genotypes located have demonstrated by different colors according to their corresponding species **Fig. 5**. Biplot in both conditions (CT & HS) showed that photosynthesis traits such as Pn, Gs, Tr, Ci, SPAD have positive association, while chlorophyll fluorescence traits (YII, NPQ, qP, ETR) have strong association. However, there is a weak relationship with SPAD, Fv/Fm, WUE, iWUE and NPQ.

Cluster Analysis

The dendrogram was generated to examine the relationships among different genotypes based on Euclidean distances, calculated by the ward's method under control and stress conditions as presented in **Fig. 6**. Inertia gain pointed to the categorization of the genotypes into three clusters in CT and HS treatment according to measuring traits. Cluster-I comprised genotypes including BGB083, BGB102, BGB113, BGB444, BGB451, BGB467, BGB472 (**Fig. 6**) characterized by Pn, Gs, E, WUE, iWUE, Ci/Ca, Y(II), qP and ETR. Cluster-II contains 50% of genotypes (BGB008, BGB027, BGB045, BGB086, BGB088, BGB089, BGB093, BGB096, BGB098, BGB103, BGB107, BGB109), however no variable showed significant association (**Table 5**). Cluster-III is grouped by BGB003, BGB009, BGB091 and BGB101 and the variables Pn, Gs, E, WUE, iWUE, Ci/Ca, Y(II) and ETR are most significantly associated with this cluster (**Table 5**). Furthermore, based on the relative importance of PCA communalities cluster scores, we were able to classify each cluster as sensitive or tolerant to heat stress as shown in **Table 5**. Cluster-III was assumed to be heat stress tolerant based on the highest cluster score calculated based on the relative importance

of the physiological traits. As a result, *S. commersonii* (BGB003, BGB009), *S. tuberosum* 2x (BGB091), and *S. chacoense* (BGB101) were designated as tolerant because they retained most of the stress tolerant indicators under adverse conditions.

Discussion

During heat stress, the photosynthesis reduction is linked to reduced antenna pigments in chlorophyll content (Camejo et al., 2006). Furthermore, changes in chloroplast ultrastructure can have a direct impact on the state of the photosynthetic apparatus and the rate of photosynthesis (Zhang et al., 2014). HS can damage the chloroplast structure by disordering the lamellae and increase the plastoglobulus number (Gao et al., 2018). On the other hand, in our study chlorophyll content in the scale of SPAD value (**Fig. 2**) increased in heat stressed plants but however this increase does not significantly correlated with the increase other gas exchange or photochemistry parameters (**Fig. 4**) in used potato germplasm. These observations of increased SPAD values in heat stressed plants, although repeatable and consistent with the leaf color and morphology of potato plants (Tang et al., 2018), are contrary to the findings of reduced SPAD values in wheat (*Triticum aestivum*) and rice (*Oryza sativa*) plants under heat stress (Tiwari et al., 2017). Increased SPAD under HS are also contrary to the findings of decreased chlorophyll content (based on extraction with acetone and spectrophotometric readings) under mild heat stress reported in the potato cultivar 'Desiree' (Hancock et al., 2014) and an accession of the wild species *S. chacoense* (Reynolds et al., 1990). The reason for this inconsistency remains unknown, but it may be related to the differences in heat stress temperature conditions, measurement methods and used potato germplasm among the referred studies. Additionally, BGB101 and BGB103 slight reduction in chlorophyll content is consistent with (Hancock et al., 2014). This demonstrates the genotypic response of potato cultivars to heat stress and suggests that multiple genotypes have to be used to investigate genetic diversity.

Photosynthesis serves as a global sensor of environmental stress (Biswal et al., 2011) and is highly sensitive to high temperature (Berry and Bjorkman, 1980). Considering the objective of the study, the most distinctive observable fact is a shift in the optimum temperature of photosynthesis (Berry and Bjorkman, 1980; Yamori et al., 2014) as the growth temperature changes by adaptive process explained by (Havaux, 1993). The higher rate of net photosynthesis rate of studied wild germplasm in heat stress temperature (**Fig. 2**) observed and similar results reported for the heat -resistant potato genotypes such as Desirée and Norchip (Hancock et al., 2014) and summarized that heat-tolerant genotypes can longer maintain their photosynthesis at a higher level under elevated temperatures.. The increase in net photosynthesis rate indicated that the chloroplasts did not suffer damage and suggested that the genotypic response of potato cultivars to heat stress and presence of photosynthetic traits genetic diversity. Moreover, potato tubers are stem-derived organs which act as sink of starch produced by photosynthetically active source tissues (Hastilestari et al., 2018). Hence, higher rates of the Pn suggested that the presence of potential genes which stimulated under HS rather than CT. But this mechanism is contradicted in other agronomic crops such as wheat (Wahid et al., 2007; Shanmugam et al., 2013). The difference in response to heat stress in our study may be due to the completely distinct morphology and biochemical machinery of the wheat and potato plants.

Stomatal conductance plays a vital role to avoid water loss (Goufo et al., 2017) and increase the ability of a plant to regulate internal temperature under heat stress (Schabow, 2022). We found that, on average, potato plants closed stomata and decreased Gs significantly during the heat stress (**Fig. 2**). Stomatal responses to heat depended on species, however, and paradoxically, two accessions (BGB003, BGB109) opened stomata and significantly increased Gs (Supplementary **Table 1**). Stomatal closure during heatwaves follows stomatal optimization theory (Cowan et al., 1977), and existence of strong significant correlation with Pn suggest us that the studied germplasm has the ability to create a balance between the maximizing the CO₂ uptake to produce sugar for sink tissues. Whereas, in this study, 55% of used accessions (BGB003, BGB008, BGB009, BGB027, BGB086, BGB088, BGB089, BGB091, BGB093, BGB098, BGB101, BGB109, BRS-BEL) observed to be increasing transpiration rate to cooling the leaves by latent heat due to higher transpiration rates (Zhang et al., 2016) under the HS. There is also significant positive correlation present with Pn and Gs which explicit that potato germplasm with increasing photosynthesis and transpiration, tries to maintain its metabolic activity and cool its leaves, but it comes at the cost of water loss, which is compensated by daily manual irrigation. These findings also agrees with the findings of (Berry and Bjorkman, 1980) who reported that transpiration is temperature dependent. This behavior is also in agreement with (Ullah et al., 2020; Bokshi et al., 2022), who observed an increase in transpiration rate under HS in tomato genotypes. Overall, *S. chacoense* recorded a decrease in transpiration rate, which shows the intraspecies differences between genotypes in transpiration may be due to their differences in morphology. Plants that are able to regulate their internal CO₂ levels under heat stress conditions are more likely to be successful in survival and reproduction, and therefore, contribute to genetic diversity in the plant community (Wollenweber et al., 2003; Shanmugam et al., 2013; Mendanha et al., 2020). This highlights the importance of considering the role of C_i/C_a in screening potato germplasm under heat stress. Since, C_i/C_a varies throughout the day, it is necessary to understand correlation of gas exchange parameters effects on C_i/C_a variations (Tan et al., 2018). As we observed in this screening trail, genotypes belong to cluster-3 (BGB003, BGB009, BGB091, GB093, BGB101, BRS-BEL) showed relatively higher Gs values along with high Pn under heat stress, giving high C_i/C_a ratio. This indicates the less resistance to CO₂ entry into intercellular spaces (Sun et al., 2023). Therefore, these results suggest the use of C_i/C_a ratio to identify the heat tolerant germplasm but only combing with other gas exchange parameters such as CO₂ consumption and availability traits (Ji et al., 2022).

In general, rising temperatures cause an increase in WUE and iWUE, but this increase is not always related to increased crop productivity because it only gives plant breeders and physiologists an estimate of how efficiently the plant uses available water under the studied stress or high temperatures (Kilemo, 2022). In our study, almost all used germplasm showed an increase in the WUE and iWUE (Supplementary **Table 1**). However, it is also important to note that they are negatively significant linked with the other gas exchange and chlorophyll fluorescence parameters (**Fig. 4**). Hence, we can hypothesize that our germplasm is a water-saving strategy by closing stomata but transpiration rate where not reduced because of regular availability of daily irrigation. Genotypes with higher rates of WUE and iWUE also indicate the use of survival strategies varying intra and inter species. Some species may prioritize water

conservation at the expense of photosynthesis, while others may try to maintain photosynthetic activity even at the expense of water loss in the form of higher transpiration rates (Ulukan, 2008).

Concerning photochemistry, in the thylakoid membranes, photosystem II is the most vulnerable component to damage. As a result, the primary effect of abiotic stress is to make PSII susceptible to photoinhibition (Li et al., 2020). From the gas exchange parameters, specifically photosynthesis rate is higher (**Fig. 2**). The positive correlations of YII, qP, ETR, and Fv/Fm with gas exchange parameters (such as Gs, Pn, and E) suggest that as photosynthetic activity increases, the efficiency of photochemical reactions in PSII also increases. This indicates that the plant is effectively using absorbed light energy for photosynthesis, resulting in higher rates of CO₂ uptake (Pn) and efficient water vapor exchange (E) through the stomata. From this study it is also observed that in wild potato germplasm demonstrate positive correlation among the photochemical quenching parameters which indicate that these parameters are closely related and collectively reflect the effective functioning of PSII and efficient electron transport during photosynthesis.

In this study, accessions belonging to distinct species group showed declining trend, in other words, which is obvious due to the presence of significant negative correlation between NPQ and photochemical quenching traits (**Fig. 4**). This indicates that the potato germplasm is efficiently utilizing the absorbed light energy for photosynthesis, reducing the need for protective mechanisms against excess light and avoiding the photoinhibition to protect the photosynthetic machinery (Mauro et al., 2011).

Taking these facts into account, we used possible photoinhibition indicators in this study to estimate the diversity of potato germplasm to tolerate heat stress. The Fv/Fm ratio was the first important parameter derived from the Kautsky curve (Krause, 1988), and it later became a key parameter for detecting PSII photoinhibition caused by a stress factor (Krause and Weis, 1991). The ratio Fv/Fm represents an estimate of maximum photochemical efficiency of PSII and is used to detect PSII reaction center function loss (Öquist et al., 1992). Fv/Fm values typically range between 0.75 and 0.85, and this ratio is proportional to photochemistry's quantum yield (Cha-um et al., 2006).

BGB003 of *S. commersonii*, BGB093 of *S. tuberosum* 2x, and five accessions of *S. chacoense* (BGB086, BGB101, BGB109, BGB467, BGB472) fall within the above-mentioned ratios under heat stress. A decrease in this ratio is thought to be a good indicator of photoinhibition (Pradawet et al., 2023). As shown by 60% of assessed germplasm, whereas BGB107 and BGB113 were the most susceptible to heat stress genotypes due to photoinhibition. It can be caused by two different processes, first is a decrease in the rate constant of PSII photochemistry caused by damage to the PSII reaction centers and/or an increase in the rate constant of non-radiative excitation energy dissipation (Kitajima and Butler, 1975). However, the decrease in Fv/Fm ratio is not always associated with photoinhibition, but it is also regarded as an indicator of PSII photoinactivation, which can occur due to the closure of PSII reaction centers or other processes such as thermal dissipation of absorbed light (Malnoë, 2018).

In current study, accessions BGB113 (*S. chacoense*), BGB008 (*S. commersonii*) and BGB089 (*S. tuberosum* 2x) showed minimum values for effective photochemical yield (YII) in quenching analysis.

Also, provides relatable data on the electron transport rate (ETR). In fact, this declining trend in YII along with ETR values talks about the inactivation of PSII which function as photoprotection by adjusting the photosynthetic photon density under heat stress conditions (Genty et al., 1989; Krause and Weis, 1991). The results of Coefficient of photochemical quenching (qP) were also in accordance with the mechanism mentioned above because the same genotypes which showed the minimum effective photochemical yield, also maintains the maximum proportion of closed reaction center under heat stress.

The PCA under control and heat stress conditions (**Fig. 5, Table 4**) explain 46.60% of variability by PC1 in studied germplasm also suggesting the same results that the variability present in the germplasm is due to the same traits which were found significantly correlated under HS. Hence, gas exchange traits such as (Pn, Gs, E, C_i/C_a , along with Y(II), qP and ETR are worth to use for screening the wild potato germplasm under heat stress conditions. However, Fv/Fm ratio could be used as a stress indicator instead of explaining variability. Furthermore, studied germplasm were divided into three clusters based on their response measured as the studied variable. Under studied conditions (**Fig. 5**), cluster 3 contain 18% of total genotypes, which can be considered as tolerant group on the base cluster score calculated by the relative importance of traits from significant PC because genotypes belong to this group were higher in net photosynthesis rate (Pn), maintained the stomatal conductivity, leads to higher transpiration rates (E), intracellular and ambient CO₂ concentration assimilation was near to 0.7 to control and also this group genotypes showed higher effective photochemical yield which explains by the higher electron transport rates and higher proportion of opened reaction center (qP) and efficient use of light energy. Additionally, less heat dissipation was observed in genotypes belonging to cluster 2. Therefore, our data suggest that under heat stress conditions, these genotypes are more efficient in transferring the light excitation energy to CO₂ fixation, thus producing a higher photosynthetic carbon gain. Contrastingly, the highest NPQ values measured in the low performing genotypes (BGB008, BGB089, BGB096, BGB107, BGB113) correspond to more intense thermal energy dissipation, leading to lower CO₂ assimilation.

Conclusion

Photosynthetic activity is a key factor to assess under heat stress in wild potato this study found significant variation for HS tolerance among potato species and genotypes based the positive correlations between and with heat stress environment of photochemical parameters and gas exchange, along with the negative correlation with NPQ, promote a more efficient and productive photosynthetic system in potato plants. This can lead to increased tuber formation, enhanced yield, and improved stress tolerance, all of which are critical for successful potato breeding and high-quality crop production.

Declarations

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Author Contributions: Conceptualization; Methodology: IB, Software: Validation: WB; Formal analysis: IB, WB, RN; Resources: GH, CC, JH, GB; Data curation: IB and RN; Writing-original draft preparation: IB; Writing-review & editing: IB and RN; Supervision: GH; Project administration: GH and CC. All authors have read and agreed to the published version of the manuscript.

Declarations

I, Ikram Bashir, the corresponding author, declares 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 have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

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Tables

Tables 1-3 not available with this version

Figures

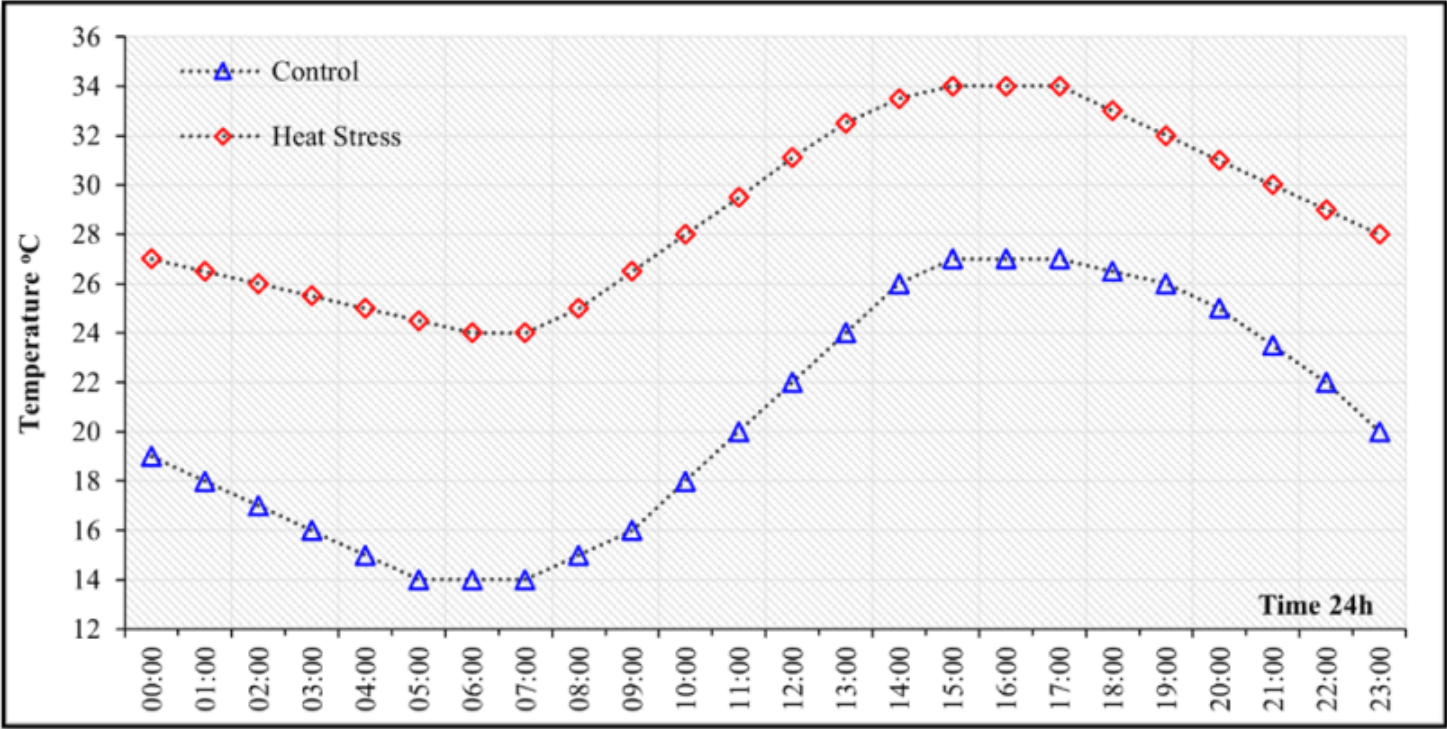


Figure 1

Growth chambers control and heat stress temperature environmental conditions 2021.

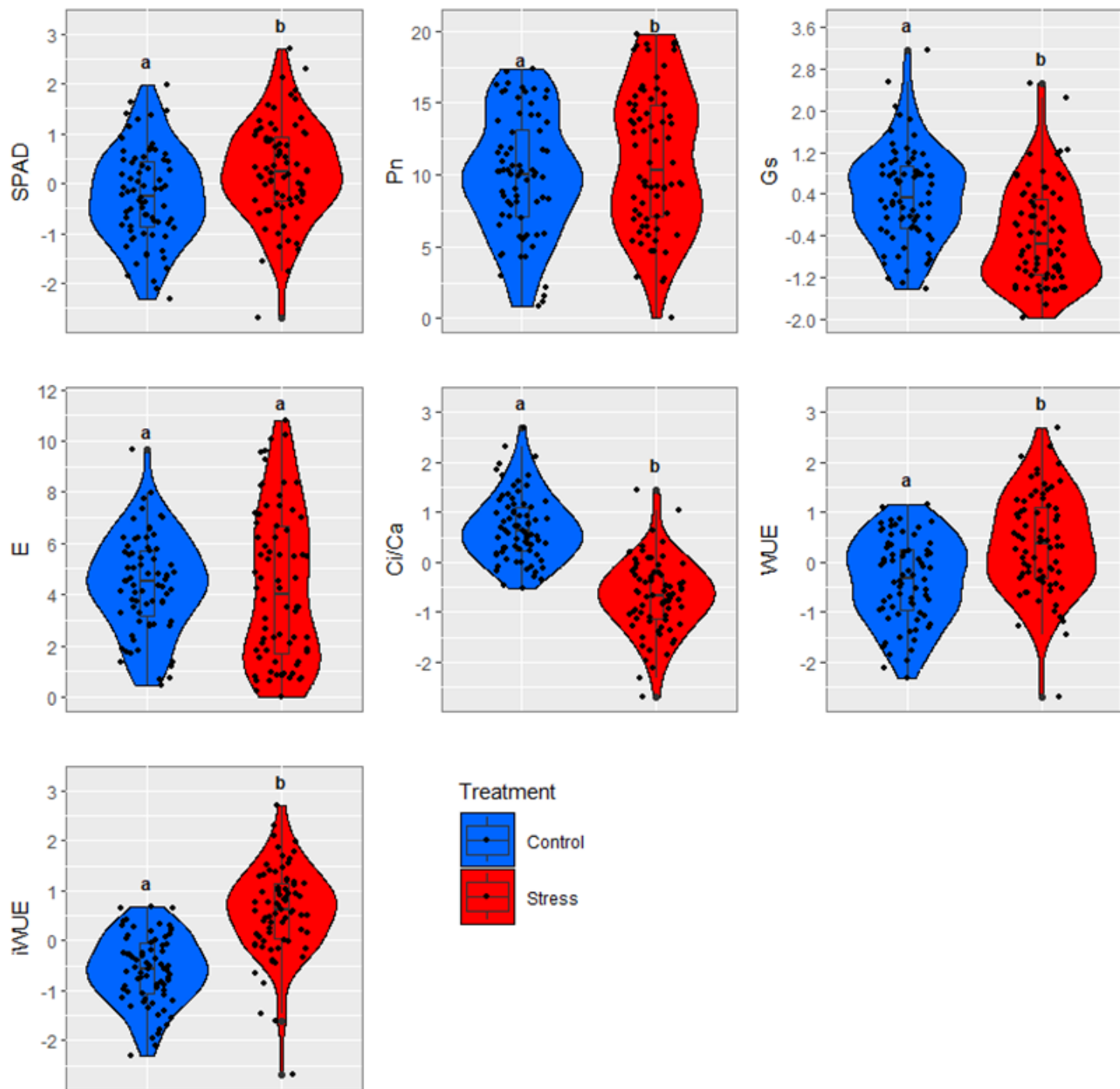


Figure 2

Mean comparison of gas exchange traits among 24 genotypes of potato (*Solanum*, Solanaceae) under control, CT, and HS stressed condition.

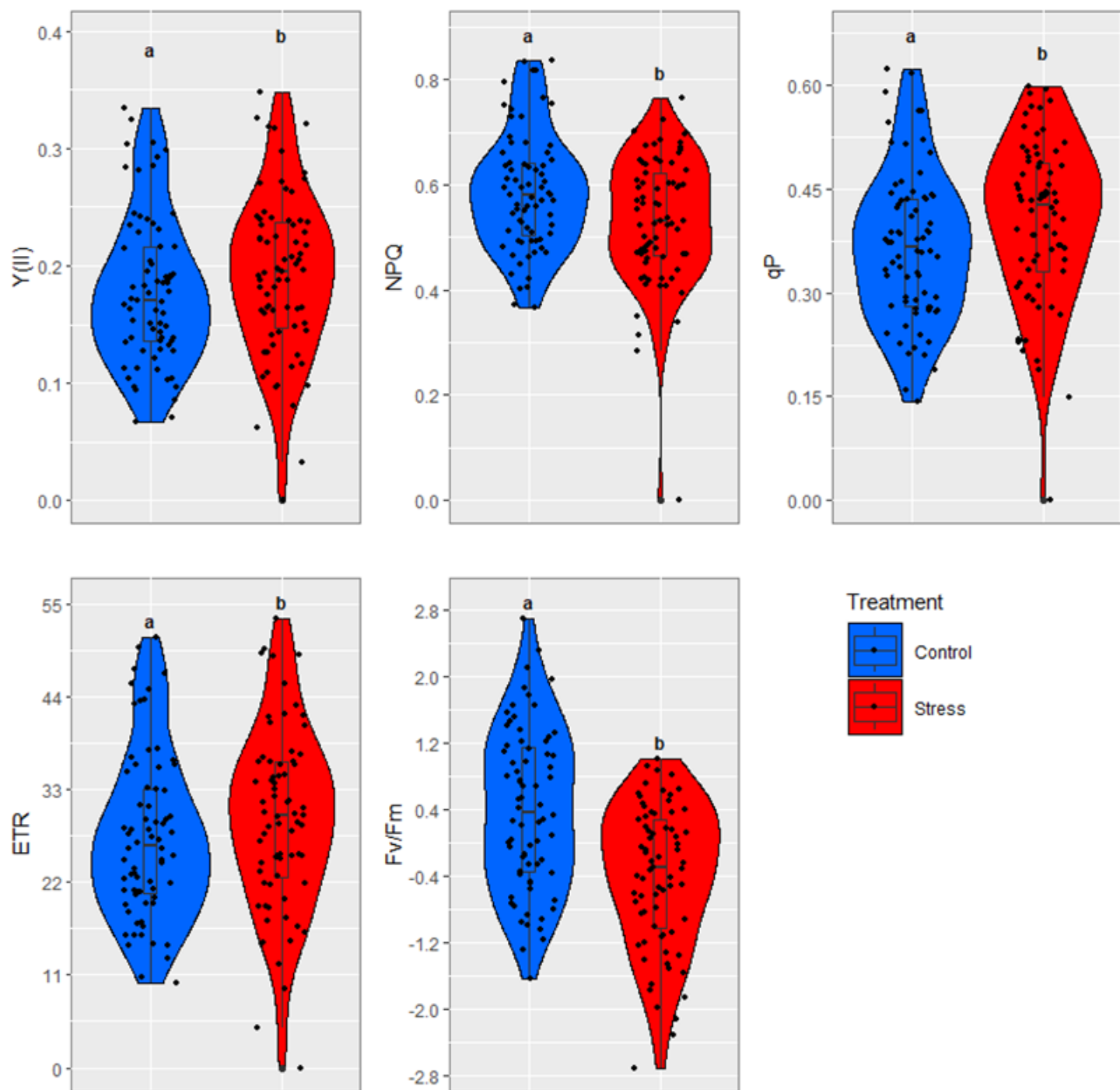


Figure 3

Mean comparison of photochemistry traits among 24 genotypes of potato (*Solanum*, Solanaceae) under control, CT, and HS stressed condition.

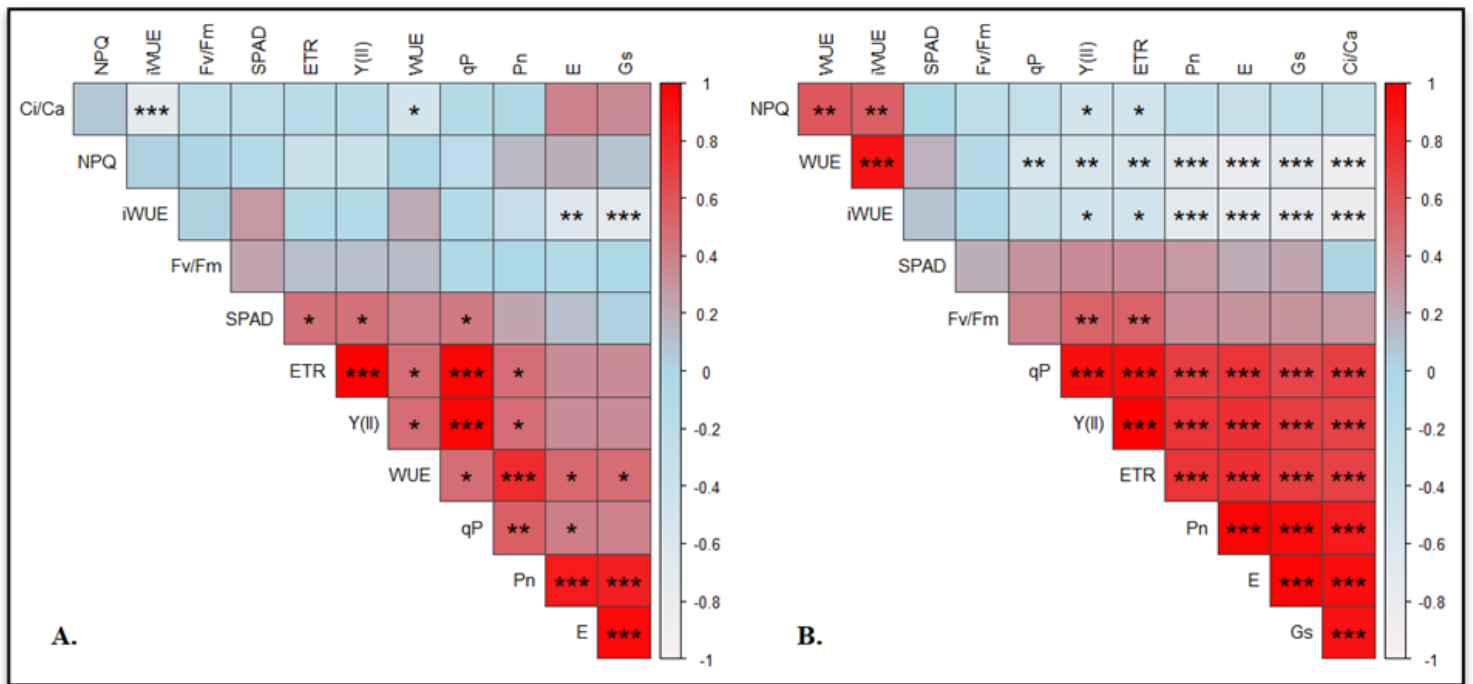


Figure 4

Correlation between different variables of chlorophyll fluorescence and photosynthesis traits in 24 potato (*Solanum*, *Solanaceae*) genotypes leaves in germplasm from Embrapa Potato Genebank. A. Control treatment (CT) 14-27°C, and B. Heat stress (HS) 24-34°C. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

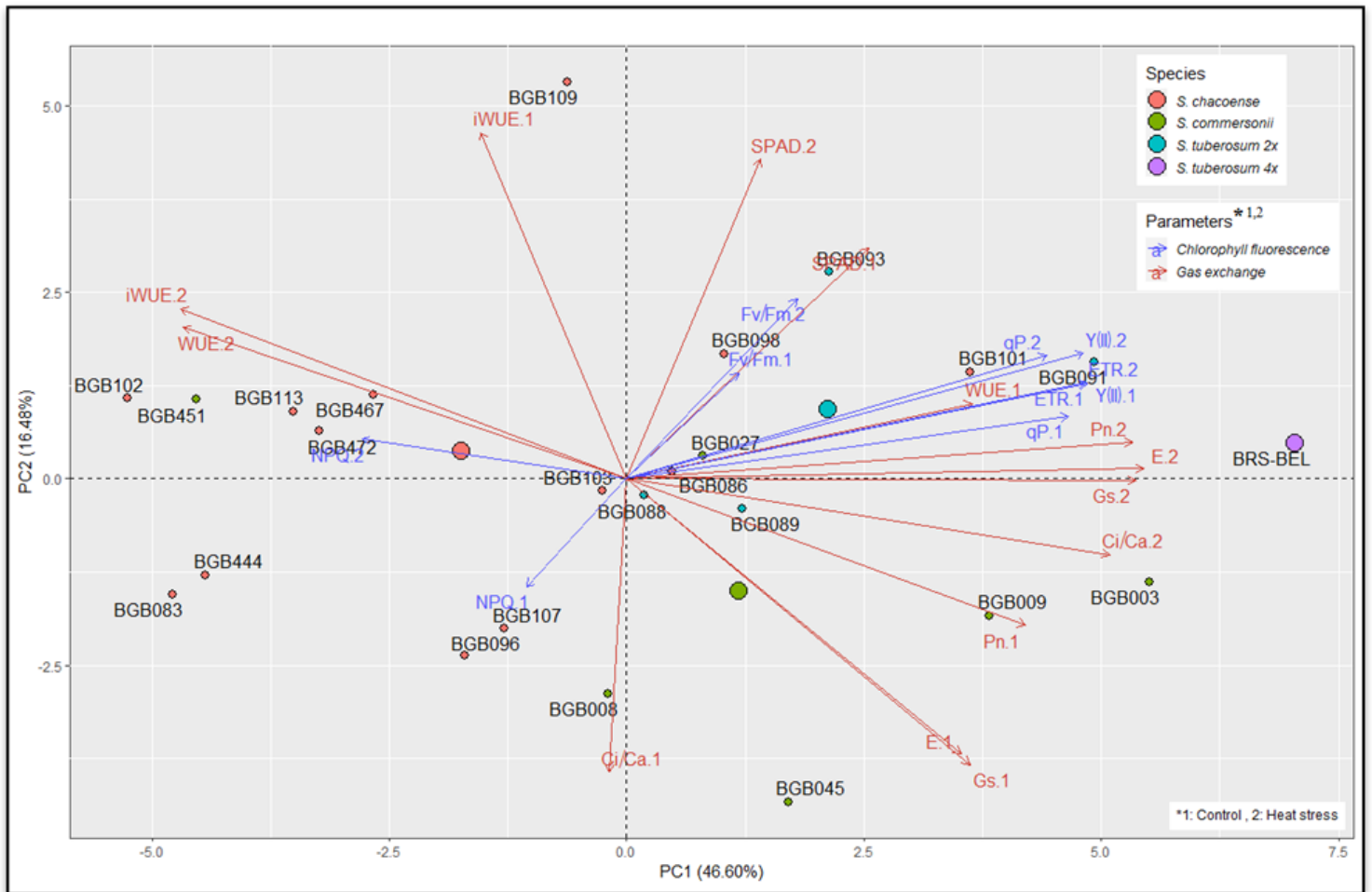


Figure 5

Dispersion of 24 potato (*Solanum*, Solanaceae) genotypes from Embrapa Potato Genebank by principal component analysis evaluated under control temperature (CT) and heat stress (HS) conditions, for the SPAD chlorophyll Index (SPAD), net photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (E), water use efficiency (WUE), intrinsic water use efficiency (iWUE), intracellular and ambient CO₂ assimilation ratio (Ci/Ca), effective quantum yield of PSII (YII), nonphotochemical quenching (NPQ), Coefficient of photochemical quenching (qP), maximum efficiency of PSII (Fv/Fm).

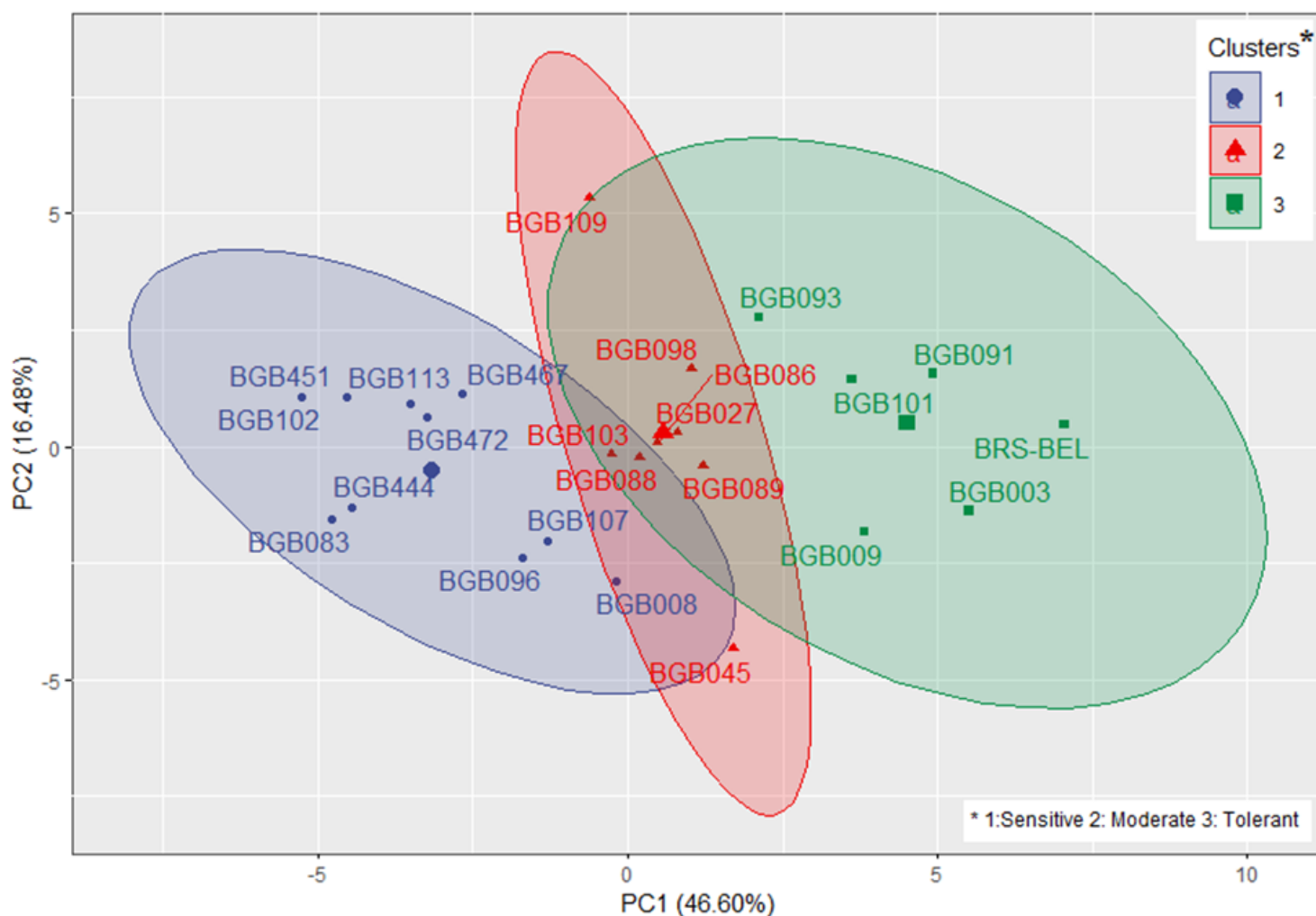


Figure 6

Cluster Plot depicting relationship between 24 potato (*Solanum*, Solanaceae) genotypes from Embrapa Potato Genebank under control and heat stress conditions.

Supplementary Files

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