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Evaluation of desirable characteristics of wild potatoes for the improvement of cultivated potato

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Evaluation of desirable characteristics of wild potatoes for the improvement of cultivated potato

Tese apresentada ao Programa de Pós-Graduação em Agronomia da Faculdade de Agronomia Eliseu Maciel da Universidade Federal de Pelotas, como requisito parcial para obtenção do título de Doutor em Ciências (área do conhecimento: Fitomelhoramento)

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Às minhas filhas,

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Resumo

BASHIR, Ikram. **Avaliação de características desejáveis de batatas silvestres para o melhoramento da batata cultivada**. Orientador: Gustavo Heiden. 2022. 153F. Tese de Doutorado (Programa de Pós-Graduação em Agronomia - Fitomelhoramento) – Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2022.

Estresses abióticos, especialmente calor e seca, são os principais fatores que reduzem a produtividade da batata (*Solanum tuberosum*, Solanaceae) limitando a atividade fotossintética, diminuindo a produção e o particionamento de assimilados para os tubérculos. As batatas-silvestres são uma fonte natural de características valiosas para o melhoramento genético da batata, como a tolerância a estresses abióticos. Entretanto, é necessário identificar os fenótipos com base em caracteres morfoagronômicos e fisiológicos, para permitir incrementar a resiliência desse cultivo, pois as trocas gasosas, índice de clorofila, e fluorescência de clorofila são os principais fatores que influenciam a produção e atividade fotossintética. De modo a avançar na avaliação de características desejáveis das batatas-silvestres em prol do melhoramento genético da batata, está tese está organizada em quatro capítulos. O Capítulo 1 apresenta uma revisão de literatura sobre o papel promissor dos parentes silvestres da batata como um reservatório de novas características genéticas desejáveis para o melhoramento da batata. O Capítulo 2 apresenta um estudo da resposta genotípica e seleção de parentes silvestres da batata quanto características dos tubérculos em condições de estresse por calor. Duas condições de temperatura foram aplicadas: controle (14-27°C) e estresse de calor (24-34°C). Ao final do ciclo de vida das plantas, os caracteres morfoagronômicos foram analisados por meio de modelos estatísticos mistos para ranquear os genótipos de acordo com os valores genotípicos reais. O Capítulo 3 trata da avaliação sob as mesmas condições de temperatura do capítulo anterior e avalia a resposta fisiológica (taxa fotossintética - Pn), taxa de transpiração (Tr), condutância dos estômatos (Gs), concentração de CO_2 intra e extracelular (C_i/C_a), e a fluorescência de clorofila. Estas características fisiológicas foram submetidas a análises de componentes principais que permitiram agrupar os acessos expostos ao estresse de calor, permitindo reconhecer um conjunto de genótipos tolerante às temperaturas elevadas. No Capítulo 4, acessos adicionais de *S. commersonii* e *S. chacoense* e características adicionais de trocas gasosas (Pn, Tr, Gs), fluorescência de clorofila (YII, NPQ, Fv/Fm), conteúdo de clorofila a e b e de carotenóides, também foram medidos 1 dia após o estresse (DAS), 15 DAS e 35 DAS. Ao final do ciclo de vida, as amostras tiveram o conteúdo total de água e os conteúdos de matéria fresca e seca observados. Os resultados demonstram que os genótipos de *S. chacoense* foram mais tolerantes e tiveram melhor desempenho sob condições de estresse de calor. Portanto, a introdução e caracterização de genótipos com características fisiológicas de batatas-silvestres é necessária para atender os esforços de ganho requeridos em programas de melhoramento genético da batata visando enfrentar cenários previstos de incremento de calor e seca globais.

Palavras-Chave: Estresse abiótico; batatas silvestres; troca gasosa; fluorescência da clorofila; *Solanum commersonii*; *Solanum chacoense; Solanum tuberosum*

ABSTRACT

BASHIR, Ikram. **Evaluation of desirable characteristics of wild potatoes for the breeding of the cultivated potato**. Advisor: Gustavo Heiden. 2022. 153 Pages. Doctorate Thesis (Graduate Program in Agronomy – Plant Breeding) – Faculty of Agronomy Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2022.

Abiotic stresses, specially heat and drought, are the major factors reducing potato (*Solanum tuberosum*, Solanaceae) productivity by limiting the plant photosynthesis activity, reducing the production, and partitioning of assimilates to the tubers. Wild potatoes are a natural source of valuable traits for potato breeding, such as abiotic stress tolerance. However, it is necessary to identify phenotypes on a morpho-agronomic and physiological basis that could be manipulated to increase crop resilience. Thus, the morphoagronomic and photosynthetic traits of gas exchange, chlorophyll index and chlorophyll fluorescence are important to study because these factors mainly influence production and photosynthetic activity. To advance on the evaluation of desirable traits of wild potatoes for the breeding of the cultivated potato, this thesis is organized in four chapters. Chapter 1 presents a literature review on the promising role of potato wild species as a reservoir of desirable novel genetic traits for potato breeding. Chapter 2 presents a study of the genotypic response and selection of potato wild relatives for their tuber traits under heat stress. Two temperature conditions: control (14-27°C) and heat stress (24-34°C) were used. At the end of the plant life cycle morpho-agronomic traits were analyzed by statistical mixed models to rank the genotypes according to true genotypic values. Chapter 3 deals with the evaluation of genotypes grown in the same temperature conditions and their physiological traits response (net photosynthesis rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), intra and extra cellular CO_2 concentration (C_i/C_a)) and chlorophyll fluorescence traits. These physiological traits were analyzed by principal component analysis and clustering of genotypes under heat stress condition resulted in a group of genotypes tolerant to elevated temperatures. In Chapter 4, further accessions of *S. commersonii* and *S. chacoense* and, apart from gas exchange traits (Pn, Tr, Gs) and chlorophyll fluorescence traits (YII, NPQ, Fv/Fm); chlorophyll a, b and carotenoid contents, were also measured after 1 day after stress (DAS), 15 DAS and 35 DAS application. At the end of the life cycle total water content and fresh tuber weight and dry matter content were also measured. The results point out that the genotypes of *S. chacoense* were more tolerant and performed better under heat stress conditions. So, introducing the characterized wild potato genotypes, with improved photosynthesis traits and other studied physiological traits in adverse conditions, is important for potato breeding programs to meet the genetic gains efforts required to cope with anticipated increasing of heat and drought events worldwide due to climate change.

Keyword: Abiotic stress; wild potatoes; gas exchange; chlorophyll fluorescence; *Solanum commersonii*; *Solanum chacoense; Solanum tuberosum*

Lista de Figuras

Lista de Tabelas

Lista de Tabelas Suplementares

Sumário

1. General Introduction

Effects on crop production can be complex. BOYER, (1982) explained that productivity depends upon adapting plants to grow in certain climatic conditions. So, an increase or decrease in production is followed by extreme weather conditions (ELAHI *et al.*, 2022; YAN *et al.*, 2022). Over millennia the earth's climate evolved constantly (LIN; QIAN, 2022). However, the greenhouse problem threatens to change climate in an unanticipated manner. Thus, climate change represents a supplementary stress over agriculture (CHAUDHRY; SIDHU, 2022; HOFFMANN *et al.*, 2020; SHAHZAD *et al.*, 2021).

HOUGHTON (2001) was alarmed that due to global warming an observed trend of temperature increase has been 0.6^{o}C + 0.2^{o}C since 1900. Then, there will be an increase of 1.4-5.8^{o}C by the period between 1990 to 2100. The direct impacts of climate change will be the decrease in crop productivity but may vary in different regions under different climatic conditions (MCCARTHY *et al.*, 2001).

According to The Global Climate Risk Index (ECKSTEIN; KÜNZEL; SCHÄFER, 2017), Pakistan, for example, is among the top 10 countries which is or will be most affected worldwide by climate change. The studies of (FAROOQI; KHAN; MIR, 2005) projected that climate change in the country includes strengthening of monsoon circulation, increase in surface temperature, and increases in the magnitude and frequency of extreme rainfall events. Altogether, these effects will cause sea-level rise which leads to the impacts on the country's ecosystems and biodiversity; hydrology and water resources; agriculture, forestry, and fisheries; mountains and coastal lands; and human settlements and human health. On the opposite side of the globe, Climate News Network (2013) predicts that if present trends in greenhouse gas emissions continue, average temperatures in Brazil will be 3-6^{o}C higher by 2100 than they were at the end of the 20th century. So, rainfall patterns could change drastically across this country, increasing by up to 30% in the South and South-east region, while diminishing by up to 40% in the Midwest, North and North-east regions. As well as in Pakistan, the climate change scenario will ultimately increase drought stress likewise decrease agriculture production (ROCHA, 2013).

In addition, arable land is decreasing day by day due to various human activities and have resulted in saline or drought conditions. KONDRÁK *et al.* (2012) considered that 70% of world's fresh water is used by agriculture. They also estimated that 20% of world's arable land is irrigated and provides 40% of food and feed, although among this irrigated arable land, 50% suffers from salinity damage (ZÖRB; GEILFUS; DIETZ, 2019). Another study by

(JAGGARD; QI; OBER, 2010) predicted that 30% of arable land will be lost within 25 years and around half of arable land will be unavailable by 2050. Thus, increasing limited water resources is an important aspect to consider soon and changing climatic scenario is creating more challenges to the development of new crop varieties which are more vigorous and more efficient in performance (LOBELL *et al.*, 2008).

Cultivated potatoes (*Solanum tuberosum* L., Solanaceae) are derived from wild species that were widely grown in the Andean regions from Peru to Chile, including the Chiloé archipelago (JANSKY; SPOONER, 2018). The crop domestication occurred between 10,000 and 8,000 years ago, from diploid wild species (2n = 2x = 24). Because of the conquest and settlement of the American continent by European explorers, potatoes were later spread out of South America and became one of the primary pillars of global food security (PEARSALL, 2008). The first domesticated potato, *S. stenotomum* (2x), was thought to be a descendant of a diploid (2x) wild species. Autopolyploidization of early diploid landraces resulted in the Andean cultivated tetraploids (*S. tuberosum* group Andigena; 2n = 4x = 48; and *S. tuberosum* groups Stenotomum [2x] and Phureja [2x]). The domestication from the wild species belonging to the *S. brevicaule* complex included selection for underground traits such as large tubers of various shapes and colors, shorter stolons, and reduced bitter taste due to the decreasing of the tuber glycoalkaloids content. Potato genome sequencing research revealed a prodigious genetic variation and signatures of gene selection that control the domestication traits (HARDIGAN *et al.*, 2017). Following the Columbus voyages, two potato introductions were brought from South America to Europe in the 16th century, encouraging potato cultivation and the establishment of a few cultivars until the 18th century (GLENDINNING, 1983). By the end of the 18th century, a second wave of potato introduction based on long-day photoperiod adapted potato landraces from Chile (*S. tuberosum* group Chilotanum, 2n = 4x = 48) took place in Europe, taking the progenitors of modern commercial cultivars to this continent, from which it was distributed along the European colonies and allied trade partner countries worldwide.

There were no serious breeding efforts for potato improvement until the mid-1800s. Asexual propagation of clones was commonly practiced by harvesting a few tubers for the following year's planting. The famous Irish potato famine caused by late blight (*Phytophthora infestans*) eliminated most potato cultivars available that time, contributing to Europe's shrinking potato gene pool. Following the mid-nineteenth century famine, serious efforts were prompted by the collection of sexually propagated potatoes from Chile and Andean diversity centers. These collections were crossbred, as well as interspecific hybridization, with the few surviving European cultivars. True potato seeds (TPS) were obtained from naturally occurring

berries (mostly via uncontrolled self-pollination), and a selection strategy resulted in early tuberization cultivars and high-yielding clones (KUMARI; KUMAR; SOLANKEY, 2018). This chain of events increased variation available for breeders and new cultivars were released. Later selection trials on 'Garnet Chili' by Albert Breese resulted in the release of 'Early rose' in 1867. As a subject of potato improvement, Luther Burbank concentrated on an open-pollinated population. His breeding efforts resulted in the 1876 release of 'Burbank Seedling,' later known as 'Russet Burbank' in the mid-twentieth century. In the twentieth century, crossbreeding and hybridization received a lot of attention for improving potato germplasm (JANSKY; SPOONER, 2018). However, the selection associated with domestication and breeding efforts reduces crop variability due to the genetic bottleneck effect demanding constantly the quest for new sources of genetic variability.

Since potato was domesticated in tropical high altitude and warm temperate climates, it is originally well adapted to milder conditions due to its centers of origin and domestication (HAVERKORT, 1990). Partially explained by its places of origin and domestication, potato tuberization diminishes (REYNOLDS; EWING, 1989) at high temperatures above 17°C, while severe damage may occur when temperature goes below 0°C, as the potato plants are originally also frost sensitive (HIJMANS, 2003). This crop is the world's third most important plant food source for humans with annual production of 376.83 M ton and is the 8th largest crop on an area of 19.25 M hectare in the world. Pakistan and Brazil ranked 19th and 20th concerning potato production with an annual yield of 4.00 M ton and 3.85 M ton respectively. The ranking for net production value of potatoes of the two countries are 17th and 19th with a value of Thousand International $ of 0.65 million and 0.61 million respectively (FAO, 2020). When considered together, Pakistan and Brazilian potato productions accomplish for a total amount in between the production of countries as Poland or France, ranked 8th and 9th in world potato production (FAO, 2020). Based on this background, climate change poses a high risk for potato production in Pakistan and Brazil with a high impact on world's food security.

Today, over 4,800 potato varieties from 125 countries are widely distributed throughout the world, particularly in temperate, subtropical, and tropical regions (HAMEED *et al.*, 2018; PIETERSE; JUDD, 2014; SEO *et al.*, 2018). Despite the diverse cultivated gene pool, most modern-day cultivars have a limited inbred gene pool due to gene similarities with early twentieth-century cultivars. Most modern cultivars are only a few meiotic events apart from cultivars from the mid-20th century. Genetic gains in traditional/classical breeding programs are slow due to long breeding cycles, selection in a single hill, and genotype by environment interactions (ORTIZ, 2020). Thus, creating new potato cultivars by carefully selecting desired

genotypes is a time-consuming and difficult breeding task hardened by the narrow genetic basis of most of the main cultivars and advanced breeding lines currently available.

Luckily, potato genepool comprises countless landraces, primitive and modern cultivars, and their wild relatives. These genetic resources have proven to be valuable sources of novel traits in breeding programs such as disease resistance, environmental tolerance, and other agronomic traits, besides processing qualities of interests as high dry matter content and low reducing sugars content in tubers (D'HOOP *et al.*, 2008; HIJMANS, 2003; JANSKY, 2000). Wild potato species (*Solanum* sect. Petota Dumort.) are highly complex groups. These species have been used for disease resistance in breeding programs for over 100 years (HAWKES, J. G., 1958). According to WATANABE *et al.*, (2011) wild relatives of potato can tolerate diverse environmental conditions due to be widely distributed in most parts of the Americas, from USA to Mexico, through Central America to South America, occurring along the later mainly in the Andes of Venezuela, Colombia, Ecuador, Peru, Bolivia, and Argentina (HIJMANS, 2003). Another important secondary center of potato wild relatives lies along the lowlands from the Southern Cone of Southern America in Argentina, Paraguay, Uruguay and Southern and Southeastern Brazil where three species occur (*S. commersonii* Dunal, *S. chacoense* Bitter and *S. malmeanum* Bitter). Approximately 110 species are recognized by (SPOONER, 2009). Some species can tolerate below zero temperatures (*S. acaule*, *S. commersonii*, *S. malmeanum* and *S. megistacrolobum*), whereas many others are adapted to hot, dry, and semidesert conditions (*S. berthaultii*, *S. chacoense*, *S. neocardenasii*, and *S. gracilifrons*) (BASHIR; NICOLAO; HEIDEN, 2021; HAWKES, 1990).

These adaptations to a wide range of habitats have made wild potato species tolerate diverse environmental stresses and develop resistance to a broad range of pests and diseases (HAWKES, 1994; ROSS; HUNNIUS, 1986). The wild potatoes *S. acaule*, *S. chacoense*, *S. spegazzinii* and *S. vernei* were reported to have resistant genes for many viruses and pests which can be used for effective potato breeding program (LOVE, 1999; ROSS; HUNNIUS, 1986). BLACK, (1937) succeeded to introgress abiotic resistance to *S. tuberosum* from wild genotypes by successfully making a cross between *S. rybinii* and *S. demissum*. The lineages obtained were also used as ancestors of two clones developed in UK named 2814a1 and 3069d4. Another successfully story of introgressing resistance for biotic and abiotic stresses to a cultivated breeding line named as CIP-24 developed in China, was based on the pedigree of *S. stoloniferum*, *S. demissum* and *S. acaule* (ORTIZ, 2001). In European cultivars, six wild relatives were widely used for resistance genes transformation such as S. demissum for late blight and potato leafroll virus (PLRV); S. acaule for potato virus-X (PVX), PLRV, potato

spindle tuber viroid (PSTV), wart, Globodera; S. chacoense potato virus-A (PVA), potato virus-Y (PVY), late blight, Colorado beetle and tuber moth; *S. spegazzinii* for Fusarium, wart and Globodera, *S. stoloniferum* for PVA and PVY; and *S. vernei* for Globodera (BASHIR; NICOLAO; HEIDEN, 2021). Although explored in a more limited extent, than the breeding for resistance to biotic stresses, WATANABE *et al.*, (2011) focused on *S. chillotanum*, *S. jamesii* and *S. okadae* as potential breeding material for transferring drought tolerance into cultivated species.

Concerning the three potato wild relatives found in Brazil, *S. chacoense* ranked third for wild potato resistance (BETHKE; HALTERMAN; JANSKY, 2017). This species showed resistance to Colorado beetles due to the presence of foliage leptines contents (acylated glycoalkaloids) that repel insects. It can survive frosts down to -3.5ºC (LI, P. H., 1977; VEGA; BAMBERG, 1995). TRAPERO-MOZOS *et al.*, (2018) determined that this species can tolerate a temperature of 40ºC even without prior acclimatization to warm temperatures. LYNCH *et al.*, (1997) identified a dominant, single gene source of Verticillium resistance in some accessions of *S. chacoense*. This species is especially interesting because could likely be crossed with *S. tuberosum* without too much difficulty.

Another wild potato species found in Brazil is the widespread *S. commersonii*, which frequently behaves as a weedy plant (HAWKES; HJERTING, 1969). It is noted to grow near to the sea, in rocky areas and dunes, which suggests some salt tolerance. This species can survive frosts down to -5ºC (LI, P H, 1977) and is reported to reach its maximum frost resistance at -11.7ºC (CHEN; BURKE; LI, 1976). Genomic analysis revealed that this species upregulates production of galactinol synthase, which has been associated in other species with the production of raffinose oligosaccharides that can protect against osmotic stress (AVERSANO *et al.*, 2015). (BAMBERG, 1995) found that at least some accessions of *S. commersonii* flower better at high temperatures than under typical temperate growing conditions with flowering set being better when greenhouse temperatures exceeded 38ºC for several hours during the day.

Solanum malmeanum is the third wild potato specie found in Brazil. This species was historically mistakenly identified or traditionally considered as conspecific with *S. commersonii*, becoming a neglected genetic resource regarding its applied uses in breeding (NICOLAO, 2021). This species is reported to possess resistance to bacterial and verticillium wilt, ring rot, late and early blight, fusarium dry rot, hapla and cyst nematode, colorado potato beetle, potato leaf hopper, green peach aphid, potato aphid, and potato leafroll virus (PLRV) (FLANDERS *et al.*, 1992; LAFERRIERE; HELGESON; ALLEN, 1999; MICHELETTO;

BOLAND; HUARTE, 2000; RADCLIFFE; LAUFR, 1971; SIRI *et al.*, 2009). It is also reputed for its freezing tolerance and high capacity to cold acclimate (i.e., increase cold tolerance after exposure to low, non-freezing temperatures) (HAWKES, J. G., 1958; HAWKES; HJERTING, 1969; ROSS; ROWE, 1965), with studies demonstrating its resistance under negative temperatures ranging from -0.55°C to -5°C, with none or a relatively small percentage (0-20%) of the leaf area damaged (HAWKES; HJERTING, 1969; ROSS; ROWE, 1965; VEGA; BAMBERG, 1995). TU *et al.*, (2021) successfully created a frost-resistant somatic hybrid potato by fusing protoplasts from resistant diploid S. malmeanum (MLM266-2) and the dihaploid susceptible potato S. tuberosum (AC142), confirming the wild relative's potential for developing a cold resistant cultivar.

In relation to abiotic stresses, heat and drought are the most likely ones to be increased in extent and duration due to climate change, although most of the work done so far on potato wild relatives abiotic stress is related to cold acclimation and breeding tolerance to frost and freezing environments (GRIFFITH; BOESE; HUNER, 1994; IOVENE *et al.*, 2004). Thus, due to the forecast of increasingly hotter and dryers conditions worldwide intensified by the climate change, it is time to prioritize the screening of heat and drought tolerance in wild potatoes and conduct research to understand the mechanisms underlying abiotic stresses response, developing strategies to incorporate these traits into the potato breeding programs.

Heat is defined as the rise in temperature beyond threshold levels for a period of time sufficient to cause irreversible damage to plant growth and development (KUMAR *et al.*, 2018). Potato plant growth and development is severely affected by high soil and air temperatures (EWING, 1981). When a sensitive plant is exposed to high temperatures, growth conditions and environmental conditions result in a rapid and dramatic adverse change in the photosynthetic mechanisms which affect the plant at cell level (BERRY; BJORKMAN, 1980; QUINN; WILLIAMS, 1985). Many scientists agreed that during these conditions, thermal damage is associated with components of the photosynthetic system located in thylakoid membranes, most likely PS-II which involves a physical separation of the peripheral light-harvesting pigments (LHC-II) from the PS-II complexes (ARMOND; SCHREIBER; BJORKMAN, 1978; GOUNARIS *et al.*, 1984; SCHREIBER; BERRY, 1977; SUNDBY; MATTSSON; SCHIÖTT, 1992). Commonly associated with heat, drought is a frequent extreme climate condition over land characterized by plant transpiration exceeding the water intake after normal precipitation over a period of months to years. Drought is often classified into three types, meteorological drought, agricultural drought and hydrological drought (SUN *et al.*, 2022; WILHITE, 2000). The effects of droughts stress on potato crop are primarily on

vegetative growth which can be characterized as shoot length, leaf size and leaf number, photosynthetic rate (KIZILOGLU *et al.*, 2006) and tuber formation stage, the latter being considered as the most harmful effect on crop yield during drought stress, with results deteriorating tuber productivity and quality (DEBLONDE; LEDENT, 2001).

Several new ideas, approaches, and technologies have emerged recently that could affect the future direction of biotic and abiotic resistance potato breeding. Thus, there are new opportunities to harness molecular techniques in the form of linked molecular markers to speed up and simplify selection of host resistance genes (BETHKE; HALTERMAN; JANSKY, 2017). Biotic stresses are also a concerning issue in potato cultivation, to address this situation, in 2004 the first potato cultivar having resistance against late blight was developed by using the wild relative *S. bulbocastanum* (HERMSEN; RAMANNA, 1973), for example. Potato wild relatives have been also used in developing cultivars possessing resistance against abiotic stresses like heat and drought tolerance, one of the examples of this contribution is Kufri Surya, an early maturing variety with oblong tubers, white smooth skin and pale-yellow flesh. It is a progeny of Kufri Lauvkar, an early bulking variety as female parent and LT-1 as male parent made by the International Potato Centre, Lima, Peru, for lowland tropics (MINHAS *et al.*, 2006).

Overall, climate change threatens world agriculture, so we need specific approaches to meet the hunger needs of a population growing subjected to this climate change scenario. Potatoes are a staple in many countries. Doubtlessly, the increasing average temperature will result in more frequent events of heat and drought waves which will affect global potato yield and quality. To tackle these challenges, this thesis addressed how we can compete against these questions, especially the abiotic stresses of heat and drought, by screening and identifying wild potatoes that possess desirable traits to cope with increasing heat and drought conditions for potato breeding. The premise that potatoes wild populations are naturally variable and have evolved under natural selection to deal with unfavorable and unpredictable conditions, underlies the working hypothesis that the wild relatives bear genetic variability to cope with abiotic stresses that are not found in the domesticated potato primary genepool. Hence, potato wild relatives screening for morphoagronomic and physiological traits under abiotic stress conditions could reveal new sources for the discovery of adaptation desirable traits to extreme weather conditions allowing the genetic improvement towards developing climate proof novel potato cultivars.

1.1. General objective

Evaluation of desirable traits for adaptation to the abiotic stresses of heat and drought from wild potatoes conserved at Embrapa Potato Genebank to the breeding of the cultivated potato.

1.2. Specific Objectives

(1) Compile and synthetize information on the potential and applied uses of potato wild relatives for the genetic improvement of new potato cultivars.

(2) Screening of *S. chacoense*, *S. commersonii*, and *S. tuberosum* 2x germplasm accessions under heat stress condition by using mix models on tuber yield data.

(3) Screening of *S. chacoense*, *S. commersonii* and *S. tuberosum* 2x germplasm accessions under heat stress condition by accessing their response physiologically.

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Chapter I

1. Wild Potatoes: A Genetic Reservoir for Potato Breeding[1]*

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1.1. Introduction

Wild potatoes are a large genetic reservoir for potato breeding with current use and potential to provide genes for novel traits, and resistances for abiotic and biotic stresses absent in commercial cultivars. The first steps to use wild relatives in modern potato breeding started almost two centuries ago. Wild species from South America had been introduced in Europe in 1824, especially *S. commersonii* (Fig. 12.1) and *S. maglia*. Before 1920 many efforts were made to cross *S. demissum* with *S. tuberosum* and by 1932 introgression of resistance genes succeed first for late blight, later for virus (1941) and potato cyst nematode [1].

Since then, many resistance genes have been introduced into modern potato cultivars. Potatoes (*Solanum* sect. *Petota*) taxonomy is a matter of debate. Accepted wild species ranges from 107 [2] to 180 [3], and domesticated from 4 (*S. ajanhuiri*, *S. curtilobum*, *S. juzepczukii*, *S. tuberosum* group *Andigenum*) [2] to 7, being 4 of them hybrids (*S.* × *ajanhuir*, *S.* × *chaucha*, *S.* × *curtilobum*, *S.* × *juzepczukii*), 2 of them with 4 subspecies (*S. stenotomum* subsp. *goniocalyx*, *S. stenotomum* subsp. *stenotomum*, *S. tuberosum* subsp. *andigenum*, *S. tuberosum* subsp. *tuberosum*) and 1 with no subspecies (*S. phureja*) [3].

Wild potatoes occur from southwestern United States of America (38°N) to central Argentina and Chile (41°S) and the Juan Fernández Archipelago. Mostspecies are from South America with the diversity peaking at 21°S and a secondary center of diversity lies around 20°N in the central Mexican highlands [4]. They occur along a wide range of habitats from deserts to rainforests and from sea level to elevations around 4,700 m above sea level in the tropical Andean mountains. Potato wild relatives grow in sunny to partially sunny areas and in preserved or anthropogenic disturbed environments, including tussocks and grazed areas and edges or glades in temperate and subtropical forests [5].

[1] Bashir, I., Nicolao, R. and Heiden, G., 2021. Wild Potatoes: A Genetic Reservoir for Potato Breeding. In Wild Germplasm for Genetic Improvement in Crop Plants (pp. 215-240). Academic Press.

Figure 1: *Solanum commersonii*, a potato wild relative native from Southern Brazil, Argentina, Paraguay, and Uruguay. (A) Wild potato plant growing in the pampas grasslands at Chuí, Rio Grande do Sul, Brazil. (B) Wild potato flowers. (C) Wild potato berries. (D) Wild potato tubers. (Photos by Gustavo Heiden.)

1.1.1. Commercial traits

Potatoes are a high source of carbohydrates with high nutritional value providing vitamins and minerals in the form of cooked tubers and processed products such as chips and canned potatoes. Consumption heavily depends upon its appearance and taste, commonly known as quality traits according to consumer preference or commercial use. Due to the huge quantity of potato tuber yield used by the processing industry, commercial traits have a high importance. Breeding objectives of these traits depend upon the market use [6]. Quality traits are in two groups. The first refers to “external quality traits” and includes tuber size, shape, eye depth, skin color, skin smoothness, and pulp color. The second refers to “internal quality traits” such as flavor, nutrients, dry matter, and starch content, glycoalkaloids and processing traits. Quality traits are genetically controlled and further classified into three major groups such as biological traits (proteins, carbohydrates, vitamins, minerals, reduced amounts of toxic glycoalkaloids), sensorial traits (flavor, texture, color), and industrial traits (tuber shape and size, dry matter content, sugar content, cold-induced sweetening, oil absorption, starch quality).

Breeding strategies to improve quality traits are achieved by sexual hybridization or genetic engineering [7]. For instance, *S. commersonii* has high dry matter content and through negative assistant selection methods, these traits were transferred to cultivated potato [8,9]. Hybrid

populations of *S. tuberosum* and *S. commersonii* were reported to have low levels of glycoalkaloids and acceptable tuber quality [9,10]. *Solanum stoloniferum* and *S. verrucosum* have quality traits used in breeding programs and low levels of glycoalkaloids [11], while *S. chacoense* has good chipping quality traits, although high level of glycoalkaloids [12]. The wild species *S. medians, S. okadae*, *S. pinnatisectum, S. raphanifolium*, and *S. sogarandinum* possess traits which make them resistant to cold-induced sweetening, an important factor in processing industry which defines the chip frying quality after tuber storage [13,14]. Other species with noteworthy quality traits are *S. vernei* for starch and *S. stoloniferum* for ascorbic acid [15]. Unfortunately, there is a lack of focus and a large gap on exploring the genetic potential of important quality traits from wild relatives of potatoes by biofortification of micro- and macro-nutrients which are important to enhance the nutritional value of potatoes.

1.2. Abiotic stresses

Climate change is the main reason for the increase of many abiotic stresses such as heat, cold, drought, and salinity. Wild potatoes occur in several climate conditions from high temperature regions to high altitude with freezing temperatures. Plants which show resistance to cold or frost stress are also most likely found tolerant to stresses such as heat, drought, and salinity [16]. For the introgression of resistant traits, diploid potato breeding is the most widely known strategy which has been used. Nowadays, molecular techniques for the identification of resistant traits at genetic level are very robust and useful. *S. commersonii*, is an example of having high levels of abiotic resistance genes for frost as well as drought, heat, and salinity stresses [17].

1.2.1. Frost

Cold stress tolerance is the oldest goal in potato breeding for abiotic stress. Potatoes are a cool season crop, but chilling temperature has an adverse effect. This condition is commonly present at night and sudden increase in temperature during the day makes the crop more susceptible to cold injury. If the temperature goes below chilling it causes crystallization of water and other minerals within the leaf veins and leads to cold injury or leaf necrosis. Due to cold injury one can lose all the crops in short time [18]. *S. commersonii, S. demissum*, and their hybrids are reported to be highly resistant to frost stress. After overcoming barriers for introgression of genes, breeders successfully transferred genes from *S. commersonii* to commercial cultivars especially for tolerance to low temperatures [4,10,19–21]. Gene Scdhn1 has been reported by applying different low temperature treatments in *S. commersonii*, which has shown to be

resistant to frost stress. CBF gene clusters responsible for cold responses were also reported from *S. commersonii* and could be transferred to cultivated potato with deletion or duplication of gene for resistance to frost stress [22]. The highest frost tolerance assessed by ability to survive cell injury in frost killing temperature was found in *S. acaule* (100%), and *S. albicans* (100%), followed by *S. commersonii* (99%), *S. demissum* (92%), and *S. paucissectum* (92%) [23]. Wild potatoes as *S. boliviense* and *S. colombianum* were also reported to be highly resistant to frost, while *S. brevicaule*, *S. candolleanum*, *S. chomatophilum*, and *S. infundibuliforme* were fairly resistant [24].

1.2.2. Drought

Potato is a hydrophilic crop requiring water from germination or sprouting until tuberization. They are grown in various climatic conditions but are sensitive to drought due to the shallow root system [25]. Physiological parameters as photosynthetic and respiration rate and accumulation of glycoalkaloids could be affected by drought stress including reduction in tissue water content, and inhibition of cell elongation [26]. Incorporation of desirable traits from highly drought resistant *S. gandarillasii* to *S. tuberosum* by phenotyping seedling growth under drought stress was useful for initial selection of wild genotypes due to different growth behaviors [27]. Other wild relative's species as *S. acaule, S. boliviense, S. bulbocastanum, S. chacoense, S. iopetalum, S. kurtzianum, S. polyadenium,* and *S. raphanifolium* are somewhat resistant for drought stress while *S. jamesii* and *S. okadae* have been reported highly resistant [27]. *S. ajanhuiri*, *S. curtilobum*, and *S. demissum* also were somewhat resistant for drought [24]. Hybrids of *S. tuberosum* and *S. commersonii* with CBF1 (ScCBF1) gene driven by 35S promoter were evaluated for drought tolerance and results were promising [25].

1.2.3. Heat

Potato is a crop which requires an average 20oC throughout its life cycle. Increased temperature causes heat stress which affects potato crop to a large extent. Many biotic stresses are also triggered by heat stress such as pests and diseases [28]. Sprout development requires optimum 6oC with optimum stem elongation at 18oC and best temperature for tuber initiation at 20oC [29,30]. A difference of 5oC in temperature causes 1–3 weeks delay in tuberization [31]. The effects of heat stress affect tuber quality, shape, color, size, and maturity and tuber physiological characters resulting in bitter taste due to accumulation of glycoalkaloid, necrosis, brown spot, and changes in hormones for dormancy. Moreover, heat stress intervals during growth stages ultimately decrease shelf life of tuber and profit margins. Negative effects of

high temperature are inhabitation of vegetative growth, reduction in photosynthesis, and increasing in respiration, delay in tuber initiation, reduction in tuber growth, tuber disorders, shortened period of tuber dormancy, reduction in tuber dry matter content, and increased levels of glycoalkaloids [32,33]. F1 interspecific hybrids of *S. phureja* and *S. chacoense, S. berthaultii,* and *S. microdontum* [34] crossed with dihaploid *S. tuberosum* subsp. *andigenum* produced diploid hybrids later crossed with *S. tuberosum* resulting in tetraploid hybrids resistant for heat stress [35]. Other wild potatoes such as *S. boliviense*, *S. chacoense, S. iopetalum*, *S. kurtzianum*, *S. polyadenium,* and *S. raphanifolium* were somewhat resistant too [24]. Ongoing studies, prospecting accessions of *S. chacoense*, *S. commersonii*, and *S. malmeanum* from Embrapa Temperate Agriculture Potato Genebank [36], aiming the introgression of resistance to heat stress, has shown that some accessions of *S. chacoense* performed better than control (*S. tuberosum*) in a heat stress environment based on photosynthesis rate, transpiration rate, chlorophyll content, and tuber related traits.

1.2.4. Salinity

Reduction in agriculture land and shortage of fresh water for irrigation due to urbanization and climate change are possibly the main reasons to grow the potato crop on salty soils or irrigated with saline water leading to salinity stress. Although potatoes are moderately sensitive to salt and can bear salinity level approximately 2.0 dS/m [37], their tolerance depends on the soil type and saline water quality. The effects of salinity stress affect germination, causes non-uniform growth, reduction in tuber yield, and decrease quality reported up to 50% [38]. Salinity also affects leaves by accumulation of toxic compounds resulting in injury and death [39]. It also affects other physiological processes important for the tuber development and negatively affect the relative water content, leaf stomatal conductance, transpiration rate, and changes chloroplast structure causing serious damage to plants and crop loss [40,41]. Abundance of salt ions in irrigation water restrict the plants from water and mineral uptake and affect the soil structure for many years followed by inhibition of soil oxygen supply to roots [42]. *S. chacoense* showed resistance against salinity stress for plant survival and dry matter content under saline conditions [43]. *S. curtilobum, S. juzepczuckii*, and *S. kurtzianum*, were also found to be tolerant to salinity stress [44,45].

1.3. Biotic stresses

Potatoes and their pathogens have an intimate relationship since the two major events of dispersal outside South America occurred after the 16th century. Few genotypes were brought

to Europe and originated the modern breeding programs. These materials were grown for a long period to the late 18th and early 19th centuries [46,47]. However, the narrow genetic variability facilitates genetic erosion and resistance to be overcome [48], since most pathogens can mutate and acquire resistance [49]. This situation is well illustrated by the Irish Famine occurred in the late 19th century (1845–49) and caused by a severe outbreak of late blight [50]. The use of potato wild relatives in breeding programs aims to introgress genetic variability into the gene pool of the new elite potato germplasm. In the beginning of the 20th century, wild potatoes have been introduced in Europe toward the restoration of potato crop, especially with the introgression of late blight resistance from Mexican *S. demissum* [1]. The potential uses of wild potatoes have been reported by Bukasov in 1933 as "The revolution in potato breeding" [51]. However, potatoes are still vulnerable to pathogens such as bacteria, fungi, oomycete, viruses, and pests, all together responsible for 22% of yield losses [15]. Currently, potatoes are one of the most dependent crops of chemical treatment, which increases costs and causes negative impacts on agroecosystems and human food chain [52]. Cultivar resistance is always the most effective solution, but due to tetrasomic inheritance, broadening the genetic diversity base of cultivated gene pool with standard techniques progresses slowly.

1.3.1. Bacterial diseases

Control of bacterial diseases in potato fields is challenging. Chemical antibiotics have been widely employed to reduce damage, but the prolonged use led to bacterial adaptation and concerns on human health. Once a disease is disseminated into the fields, it may persist for long periods and spread. Depending on the environmental condition the damages could be severe, and growers are obligate to leave the infected areas for a quarantine period. The most widely recommended and sustainable management is planting resistant cultivars, followed by the adoption of good agronomic practices [53].

1.3.1.1. Bacterial wilt or brown rot (*Ralstonia solanacearum*)

This disease is caused by an endophyte soil-borne organism, distributed mainly in tropical and subtropical regions [54]. It infects roots from natural skin openings or lenticels and spread through the vascular system causing disfunction, wilting, and plant death [55]. Susceptible cultivars suffer losses that can exceed 50% [56]. It has a large genetic and phenotypic diversity and an ability to survive season by season [54]. The strains are classified in four broad phylotypes (I– IV), based on phylogenetic relationships and geographic origin, and in biovars [57]. Wild potatoes carrying resistance are *S. acaule*, *S. andreanum*, *S. brevicaule*, *S.*

bulbocastanum, S. candolleanum, S. cardiophyllum, S. chacoense, S. clarum, S. commersonii, S. jamesii, S. malmeanum, S. microdontum, S. pinnatisectum, and *S. sparsipillum* [19,24,58–66]. *S. candolleanum, S. chacoense*, and *S. sparsipillum*, reported as resistant to bacterial wilt, when hybridized with *S. tuberosum*, revealed undesirable high glycoalkaloid contents in the offspring [60]. Ploidy manipulation to incorporate resistance from *S. commersonii* into tetraploid *S. tuberosum* resulted in resistant clones already released [58,63].

1.3.1.2. Bacterial ring rot (*Clavibacter michiganensis* subsp. *epedonicus*)

Longevity in the soil, latency, and low cell levels, that remains undetected for up to three or four generations, make this disease difficult to control [67,68]. Tubers are infected through natural skin openings or lenticels [69]. The infection has a slow spreading into vascular tissues at early stages, then spread increases with Early blight **(*Alternaria solani*).** This is one of the most severe leaf-spotting and defoliation agents. Damage is severe when high humidity and 20°C–25°C temperatures are combined. Early cultivars are less affected than late ones, but all cultivars are susceptible and if no strategy of control is adopted, the disease could destroy a potato field in 6 weeks [92,93]. Some resistant levels to early blight are found in *S. acaule, S. acroscopium, S. berthaultii, S. brevicaule, S. bulbocastanum, S. cardiophyllum, S. chacoense, S. commersonii, S. malmeanum, S. neorossii*, and *S. raphanifolium* [66,77,94–97]. Some hybrids between *S. raphanifolium* and haploid *S. tuberosum* demonstrated high levels of resistance in field trials [97–99].

1.3.1.3. Fusarium wilt (*Fusarium* spp., especially *F. oxysporum* complex).

This disease has been considered as one of the most yields limiting [100], causing losses between 15% and 70%, if no effective control is adopted [101]. Potential sources of resistance are *S. acaule, S. brevicaule*, and *S. kurtzianum* [66].

1.3.1.4. Late blight (*Phytophthora infestans*).

This is the main oomycete disease affecting potatoes and the protagonist of the Irish Famine which caused hunger and deaths between 1845 and 1849, obligating more than 1 million people to migrate [50]. Introductions of *S. demissum, S.* × *edinense* and their hybrids with *S. tuberosum* presented levels of resistance that enabled the restoration of the crop in Europe [1]. Until the late 20th century, the A1 mating type was the predominant race outside Mexico. After 1980, a new population of the A2 mating type race has been reported to emerge and spread from

Mexico to many regions worldwide. The specific resistance was not durable since the two mating-types races are able to recombine and originate new virulence strains [49,102]. Wild potatoes with different levels of resistance are *S. acroglossum, S. cardiophyllum, S. chacoense, S. demissum, S. dolichocremastrum, S.* × *edinense*, *S. flahaultii*, *S. guerreroense, S. hougasii, S. iopetalum, S. jamesii, S. laxissimum, S. huancabambense, S. malmeanum, S. microdontum, S. morelliforme, S. oxycarpum, S. polyadenium, S. stipuloideum, S. tarnii, S. venturi,* and *S. verneii* [24,66,77,103–111]. The *R*-genes confer race-specific resistance to pathogen race and are classified from *R1* to *R11* and confer race-specific resistance to pathogen race [112,113]. *R*-genes are present in *S. brevicaule* [114], *S. berthaultii* [115], *S. bulbocastanum* [24,116–118], *S. cajamarquense* [119], *S. cardiophyllum, S. clarum, S. colombianum, S. polyadenium* [24], *S. chiquidenum* [120], *S. microdontum* [121], *S. mochiquense* [122], *S. papilla, S. stoloniferum,* [123], *S. pinnatisectum* [124], and *S. verrucosum* [125,126]. *S. cajamarquense* seems to have unique late blight resistance genes [119] as well as *S. huancabambense* which resist the AVR2 effector [103]. Pyramiding of *R*-genes by race non-specific (horizontal resistance) using major *Rpi*-genes, that are different alleles of one gene, or the same alleles (allele-dosage) into a single cultivar (multi-line) seems promising. Studies are mapping *R*-genes as quantitative trait loci (QTLs) and developing molecular-marker for field resistance [127]. Pyramiding *R*-genes by classic breeding is difficult and time-consuming as well as marker-assisted selection. Protoplast fusion was successful to introgress late blight resistance from *S. tarnii* to the cultivar "Delikat" [111]. Cisgenesis mediated by *Agrobacterium tumefaciens* resulted in many *R*-genes cloned into elite cultivars, improving durability, and broad-spectrum resistance [121,128].

1.3.1.5. Potato wart (*Synchytrium endobioticum*)

The disease is caused by a soil-borne biotrophic fungus [129], which can persist more than 30 years and justifies the classification as an A2 quarantine disease [130,131]. It is an obligate parasite, which infect tubers and develop galls, turning them unmarketable. Chemical control is not effective and only quarantine and phytosanitary measures, as well as cultivation of resistant cultivars, are efficient prevention methods. Wild potatoes reported for resistance are *S. acaule, S. acroscopicum, S. berthaultii, S. boliviense, S. brevicaule, S. bulbocastanum, S. cardiophyllum, S. chacoense, S. demissum, S. endobioticum, S. infundibuliforme, S. iopetalum, S. jamesii, S. kurtzianum, S. microdontum, S. pinnatisectum, S. polyadenium, S. raphanifolium, S. stoloniferum,* and *S. vernei* [24,66].

1.3.1.6. Verticillium wilt (*Verticillium* spp., especially *V. dahliae*).

This is one of the most important soil-borne diseases [132]. Control is difficult due the survival as microsclerotia on the soil or as mycelia in the vascular tissue or tuber remaining in the field [133]. It infects through the roots and hyphae spread efficiently in all vascular tissues obstructing them, causing wilting of leaves, chlorosis, necrosis, and premature senescence [134]. Wild potatoes with high levels of resistance are *S. berthaultii*, *S. brevicaule*, *S. candolleanum*, *S. chacoense*, *S. malmeanum*, *S. raphanifolium*, and *S. verneii* [77,135–137]. A single dominant gene in *S. chacoense* is responsible for resistance [138]. Hybrids from reciprocal crosses between *S. brevicaule*, and *S. chacoense* evaluated under greenhouse conditions, reported resistance [139].

1.3.2. Pest insects

Insects affect yield and tuber quality with global losses achieving 10%–16%. The damage can be direct, by feeding on leaves or tubers, or indirect through the transmission of viruses [140]. Trichome-mediated insect-resistance is related to glandular trichomes-types on leaves and high levels of glycoalkaloids protecting from herbivory [141,142]. The glandular trichomes can be of two types: A-type has a short stalk and a four-lobed head, which ruptures when touched releasing a phenolic fluid and polyphenol-oxidase (PPO) [143], while B-type exudes a sticky droplet [144]. There are no potato cultivars significantly resistant to the main insect pests and control usually relies on pesticides [145].

1.3.2.1. Colorado potato beetle (*Leptinotarsa decemlineata*).

Trichome-mediated resistance has been reported in wild potatoes such as *S. berthaultii* [146] and *S. polyadenium* [147]. Defense reported in *S. chacoense* is due to the high content of the glycoalkaloid leptin [148] synthesized in aerials tissues but not in tubers [149]. Resistance found in *S. acroglossum* is attributed to trichomes lacking high concentrations of glycoalkaloids [150]. Secondary metabolites may be an interesting approach for resistance and has been found in *S. acaule*, *S. acroscopicum*, *S. albornozii*, *S. berthaultii*, *S. brevicaule*, *S. boliviense*, *S. candolleanum*, *S. cardiophyllum*, *S. chacoense*, *S. chomatophilum*, *S. clarum*, *S. commersonii*, *S. demissum*, *S. infundibuliforme*, *S. jamesii*, *S. kurtzianum*, *S. malmeanum*, *S. morelliforme*, *S. neocardenasii*, *S. oxycarpum*, *S. paucissectum*, *S. pinnatisectum*, *S. piurae*, *S. polyadenium*, *S. stoloniferum*, and *S. tarnii* [24,66,141,147,151–157].

1.3.2.2. Flea beetle (*Epitrix harilana* rubia).

Resistance has been found in *S. berthaultii* [158] and *S. bulbocastanum* [159].

1.3.2.3. Green peach aphid (*Myzus persicae*) and potato aphid *Macrosiphum euphorbiae*).

Damages are caused by feeding on leaves with high concentration of carbohydrates, uptaking of nutrients from suction, cytotoxic effect of salivary secretion, and virus transmission [160]. Wild potatoes possessing some resistant level are *S. berthaultii*, *S. brevicaule*, *S. bulbocastanum*, *S. candolleanum*, *S. cardiophyllum*, *S. chacoense*, *S. chomatophilum*, *S. clarum*, *S. guerreroense*, *S. infundibuliforme*, *S. hjertingii*, *S. neocardenasii*, *S. lignicaule*, *S. medians*, *S. microdontum*, and *S. polyadenium* [161–163]. Protoplast fusion transferred resistant genes from *S. bulbocastanum* to *S. tuberosum* [164].

1.3.2.4. Mites (*Tetranychus urticae*)

Somewhat resistant levels have been found in *S. berthaultii* [158] and *S. bulbocastanum* [159]. The glandular type-B trichome which confers resistance in *S. berthaultii* has been introgressed into the cultivated potato [146,147].

1.3.2.5. Tuber worms or tuber moths.

Andean tuber moth (*Symmetrischema tangolias*), Guatemalan tuber moth (*Tecia solanivora*), and potato tuber moth or tuberworm (*Phthorimaea operculella*) are important pests at subtropical and tropical regions, causing yield losses in field but mainly during storage [165]. Along growing season, *P. operculella* lays eggs preferentially on the leaves, over tubers, or on soil next to the host plant [166]. The larvae damages through feeding, digging tunnels in tubers, stems, petioles and leaves [167], and increases the risk of infections [168]. Yield losses happen during the post-harvest, caused by weight and quality loss, and unmarketable tubers [167]. Wild potatoes as potential sources of resistance are *S. chacoense*, *S. chiquidenum*, *S. commersonii*, *S. pinnatisectum*, and *S. tarijense* [169,170].

1.3.2.6. Potato leafhopper (*Empoasca fabae*).

This pest is a severe defoliator and reduces the accumulation of reserves by affecting leaves [171]. Resistance is reported in *S. berthaultii*, *S. chacoense*, *S. commersonii*, *S. malmeanum*, and *S. polyadenium* [141,159,172].

1.3.2.7. Potato leaf miner (*Liriomyza huidobrensis*).

This highly invasive pest causes severe damage to the leaves and favors infection by *Alternaria solanii* [173]. Some resistance is reported to *Solanum chacoense* [94].

1.3.3. Nematodes

These are critical pests causing severe physiological disorders, resulting in yield losses and unmarketable tubers [174]. Chemicals treatments are realized by fumigants and non-fumigants nematicides with high monetary, environmental, and health costs. Integrated pest management can adopt crop rotations but when the infestation is high, quarantine is the only decision treatment [175,176].

1.3.3.1. Potato cyst nematodes (*Globodera rostochiensis, G. pallida*).

Potato breeding using wild relatives as resistance source against cyst nematode is an option especially since the discovery of it in *S. vernei* [177,178]. Other sources are *S. acaule, S. berthaultii, S. boliviense, S. brevicaule, S. bulbocastanum, S. candolleanum, S. cardiophyllum, S. chacoense, S. demissum, S. flahaultii, S. kurtzianum, S. malmeanum, S. megistacrobolum, S. microdontum, S. multiinterruptum, S. stipuloideum,* and *S. polyadenium* [24,66,94,179–181].

1.3.3.2. Root-knot nematode (*Meloidogyne chitwood, M. incognita*).

Some level of resistance is reported to *S. acaule, S. boliviense, S. brevicaule, S. bulbocastanum, S. candolleanum, S. cardiophyllum, S. chacoense, S. demissum, S. hjertingii, S. hougasii, S. iopetalum, S. jamesii, S. kurtzianum, S. microdontum, S. pinnatisectum, S. polyadenium,* and *S. raphanifolium* [24,182]. Resistance in *S. bulbocastanum* is controlled by a single monogene RMc1(blb), two genes R Mc1(blb) and RMctuber(blb), while *S. hougasii* and *S. stoloniferum* also have the resistance genes RMc1(hou) and RMc1(fen), respectively, but only partial resistance was introgressed into elite potato germplasm by the mean of protoplast fusion [183,184].

1.3.4. Viruses

At least 50 viruses are reported to cause diseases in potato [185], reducing yield up to 80% [186]. Primary transmission occurs through infection in growing plants as well as in the post-harvest [187]. Seed certification is constrained by virotic tubers [188] and plants grown from previously infected seed tubers (secondary infection) commonly produce unmarketable ones.

Aphids are the main vectors spreading virus in a circulative non-propagative manner such as for PLRV, PVA, PVV, and PVY, while PVX is only transmitted by contact as propagative manner, and PVM and PVS can be transmitted in both ways [187]. Breeding for resistance to viruses started in Europe by mid-1900 with *S. stoloniferum*, and since then many wild potatoes have been characterized for resistance to diverse strains. Symptoms include mosaic pattern on the leaves, leaf drops as well as stem necrosis [187].

1.3.4.1. Alfalfa mosaic virus (AMV)

Resistance found in *S. mochiquense*, *S. neocardenasii*, *S. neorosssii*, and *S. paucissectum* [189].

1.3.4.2. Potato spindle tuber viroid (PSTVd)

Resistance found in *S. acaule*, *S. berthaultii*, *S. candolleanum*, *S. chacoense*, *S. guerreroense*, *S. hjertingii*, *S. stoloniferum*, and *S. sucrense* [24].

1.3.4.3. Potato virus A (PVA)

Resistance found in *S. chacoense*, *S. hougasii*, *S. maglia*, and *S. stoloniferum* [190].

1.3.4.4. Potato leafroll virus (PLRV)

Resistance found in *S. acaule*, *S. acroscopicum*, *S. andreanum*, *S. boliviense*, S. *brevicaule*, *S. brevicaule*, *S. brevidens*, *S. candolleanum*, *S. chacoense*, *S. demissum*, *S. flahaultii*, *S. gourlayi*, *S. infundibuliforme*, *S. kurtzianum*, *S. malmeanum*, *S. nerossii*, *S. maglia*, *S. mochiquense*, *S. piurae*, *S. polyadenium*, *S. raphanifolium*, *S. stenophyllidium*, *S. stipuloideum*, *S. stoloniferum*, *S. trifidum*, and *S. verrucosum* [24,66,77,96,106].

1.3.4.5. Potato virus M (PVM)

Resistance found in *S. brevicaule*, *S. boliviense*, *S. brevidens*, *S. chomatophilum*, *S. infundibuliforme*, *S. maglia*, *S. raphanifolium*, and *S. stoloniferum* [106].

1.3.4.6. PVS (Potato virus S)

Resistance found in *S. berthaultii*, *S. boliviense*, *S. brevicaule*, *S. laxissimum*, *S. lignicaule*, *S. maglia*, *S. michoacanum*, *S. multiinterruptum*, and *S. stoloniferum* [106].

1.3.4.7. Potato virus X (PVX)

Resistance found in *S. acaule*, *S. ajanhuiri*, *S. albornozii*, *S. berthaultii*, *S. brevicaule*, *S. chacoense*, *S. commersonii*, *S. demissum*, *S. guerreroense*, *S. iopetalum*, *S. jamesii*, *S. kurtzianum*, *S. maglia*, *S. mochiquense*, *S. neorossii*, (*S. sparsipilum*), *S. sucrense*, *S. tarijense*, *S. tarnii*, and *S. vernei* [24,66,77,94,189–195].

1.3.4.8. Potato virus Y (PVY)

Resistance found in *S. acaule*, *S. acroscopicum*, *S. andreanum*, *S. berthaultii*, *S. boliviense*, *S. brevicaule*, *S. brevidens*, *S. candolleanum*, *S. cardiophyllum*, *S. chacoense*, *S. chomatophilum*, *S. demissum*, *S. ehrenbergii*, *S. guerreroense*, *S. hougasii*, *S. iopetalum*, *S. jamesii*, *S. kurtzianum*, *S. multiinterruptum*, *S. neocardenasii*, *S. pinnatisectum*, *S. stenophyllidium*, *S. stoloniferum*, and *S. tarnii* [24,59,66,94,106,111,189,190,194,196,197].

1.3.4.9. Tobacco Rattle Virus (TRV)

Resistance found in *S. paucissectum* [189].

1.4. Hurdles to be overcome

There are plenty of documented cases on the wild potatoes as a rich source of novel commercial traits and resistance for abiotic and biotic stresses. However, introgression of these traits into commercial cultivars is challenging. For the past decades, breeders have become focusing on the introgression of diploid wild germplasm into cultivated potato at the diploid level but in doing so there are many hurdles and barriers which obstruct the goal of achieving the tetraploid level for cultivar development [198]. Although wild potatoes are important for genetic improvement, some genetically based biological barriers prevent the introgression [199,200]. Common barriers are prezygotic barrier and cytoplasmic-genetic male sterility in which either pollen or style are incompatible [98,201,202]. Prezygotic barriers can be overcome by reciprocal crosses. Endosperm balance number is the most important postzygotic barrier. It affects the endosperm development and production of viable seeds. For normal endosperm development, it is important to keep the 2:1 maternal: paternal ratio of endosperm balance factors [203]. Wild potatoes could be 2x/1EBN, 2x/2EBN, 4x/2EBN, 4x/4EBN, and 6x/4EBN based on their ability to hybridize [2]. If EBN number does not match in interspecies crosses, abnormal development of endosperm, and inviable seed production occur. Due to different ploidy levels and EBN, it is difficult to directly cross wild potatoes with the tetraploid cultivars.

This barrier could be overcome by different techniques such as bridge crosses, 2n gametes, somatic fusion, ploidy manipulation, and sexual polyploidization [204]. In ploidy manipulation technique, doubling of chromosome results in doubling of ploidy and EBN. This scheme could be done by somatic doubling of 2x and by 2n gamete formation resulting from the failure of meiosis to reduce chromosome number [205,206]. It is also possible to manipulate the ploidy level and EBN through bridge crosses [207]. This scheme has potential to introgress tertiary genepool species to the cultivated species [208,209]. Other strategy to overcome incompatibility is somatic fusion followed by in vitro plant regeneration which is useful for producing hybrids such as somatic fusions between the 2x, 1EBN species *S. bulbocastanum* and 4x, 4EBN cultivated potato [183]. Other techniques to overcome the biological barriers are mentor pollination and embryo rescue. In mentor pollination, compatible species pollen is applied 1 or 2 days after pollinating with an incompatible species to minimize the premature fruit drop [198].

1.5. Future of wild potatoes in breeding

There is probably no other crop that has such a diversity of species distributed throughout its wild gene pool such as potatoes. Potato genepool has, more than 100 species that have evolved in many environmental conditions and provide a rich source of desirable agronomic traits as resistance for diverse biotic and abiotic stresses, and tuber quality traits that can be introgressed into cultivated potato [2,3,210,211]. Food demand by 2050 are estimated to be 70% more than required by 2006, considering a population rise to 10 billion people [212]. Potatoes are the crop that mostly produces calories per area and 2008 was considered the International Year of the Potato [213] due its important role to the economy and world food security [214]. Cultivated potato is a tetraploid and largely heterozygous crop, and its tetrasomic inheritance makes breeding through traditional crossbreeding a challenge [215,216], in the other hand, 70% of wild potato species are diploid [2]. Pre-breeding is an essential step to incorporate traits from wild relatives for the improvement of new commercial cultivars, as well as to delivering wild potato diversity in a readily manageable form. Because the genetic base of modern potato cultivars is narrow, breeders are looking for wild potatoes with more interest in Genebanks. Unfortunately, much of species are under characterized, which makes it difficult to use this germplasm efficiently. The advance of technologies such as application of omic-scale for gene discovery could accelerate introgression of interesting traits [217]. The genome of the cultivated potatoes and some of its wild relatives [218] intensified the whole-genome level studies. Genomic analysis makes it possible to find genes encoding valuable agronomic traits

such as resistance for several abiotic and biotic stresses, nutritional compounds, vitamins as even yield traits [219–221]. Furthermore, genomic analysis also supports potato breeding programs for increasing genetic gains [222] Biotechnological tools are being used to transform some traits of potato germplasm. Transcription activator-like effector nucleases (TALENs) to knockout *VInv* improving cold storage and processing traits for the commercial variety Ranger Russet [223]. CRISPR-Cas9 is an alternative tool to put desirable genes into elite germplasm, for example, increases the amounts of beta-carotene to provide a richer supply of vitamin A [224], reduce herbicide sensitivity phenotype [225], causes the complete abolition of the steroidal glycoalkaloids α- solanine and α-chaconine accumulation in potato [226], or transform diploid wild potatoes from self-incompatible to self-compatible [227,228].

Recently, the efforts increased to transform tetraploid potato at diploid hybrid potatoes that are propagated by true potato seeds, as advantage to avoid virus transmissions and reduce operational production costs. Along more than 100 species, around 70% of wild potato relatives are diploid but are self-incompatible. Tetraploid potatoes need to take more than 20 generations to have 99% of homozygosity, whereas diploid potatoes can reduce heterozygosity by 50% each generation. By this way, gene fixing becomes easily at hybrid diploid level compared to tetraploid level, and marker-assisted selection could accelerate the introgression of desirable traits [8,9]. This approach is discussed by many research groups. In *S. chacoense* a gene that allows self-compatible fertilization by response of alleles *Sli* was identified [229]. The discovery of a self-compatible gene made it possible to create an inbred line diploid potato [230,231]. Particularly, *S. commersonii* (Fig. 12.1] is the first wild potato which had the whole genome sequenced, what makes possible to exploit more genes families [17,220,221,232]. Nowadays, with the development of several new biotechnological techniques, the potential of using wild potatoes for breeding the cultivated potato is more promising than ever.

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Chapter II

2. Genotypic response and selection of potato germplasm under heat stress[2*]

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2.1. Abstract

The genetic diversity of crop wild relatives is a rich source of valuable genes for plant breeding. Potato wild relatives are an important potential source for breeding programs focused on heat resistance due to their wider adaptability to different climatic conditions. Wild potato accessions from Embrapa (Brazilian Agricultural Research Corporation) Potato Genebank were assessed and compared to dihaploid and tetraploid cultivated genotypes by measuring tuber yield-related traits and then by analyzing through mixed models using the restricted maximum likelihood (REML)/best linear unbiased predicted (BLUP) procedure under heat stress. So, the present study aimed to select the most productive wild potato genotypes under two ranges of temperatures by investigating adaptability and stability of parameters through mixed modelling. Twenty-one genotypes comprising 17 wild potatoes (thirteen diploid *Solanum chacoense*, one triploid *S. chacoense* and two diploid *S. commersonii*), four dihaploid *S. tuberosum* and one tetraploid commercial cultivar of *S. tuberosum* (BRSIPR Bel) were evaluated under favorable crop temperature and heat stress conditions using a randomized complete block design. Significant differences were observed for the effects of genotypes and the G×E interaction. Broad sense heritability ranged from 0.24 to 0.59. Genotypic variance was the largest component of phenotypic variance, followed by environmental variance and interaction variance. We observed the highest genotypic correlation by dry matter content. Accuracy of selecting wild genotypes was high for all traits. The genotypes BGB088 (dihaploid *S. tuberosum*) and BGB113 (diploid S. chacoense) performed as the best ones in most of the studied traits under heat stress. These genotypes show better stability (HMGV), adaptability (RPGV and RPGV*GM), and stability and adaptability of genetic values (HMRPGV and HMRPGV*GM) under high temperature by mixed model methodologies. Conversely, BGB009 and BGB045 (diploid S. commersonii) and BGB086 (triploid *S. chacoense*) showed consistency in ranking among the ones last for genotypic values for all methodologies. Thus,

[2] Bashir, I., Nardino, M., Castro, C.M. and Heiden, G., 2022. Genotypic Response and Selection of Potato Germplasm Under Heat Stress. Potato Research, pp.1-20. https://doi.org/10.1007/s11540-022-09573-w

we concluded that BGB088 and BGB113 are promising genotypes of interest to further studies for providing higher tuber yield under heat stress or non-favorable potato crop environmental conditions. These genotypes should be further assessed in efforts to evaluate the crossability and proceed introgression essays towards broadening the genetic basis of potato crop available for breeding to achieve more resilient cultivars under abiotic stress conditions.
Keywords: Crop wild relatives; G ×E interaction; Mixed models; REML/BLUP; *Solanum*

2.2. Introduction

Potato is an important heat sensitive, temperate region, non-grain, high demand cash crop grown all over the world (HAVERKORT; STRUIK, 2015; LIZANA *et al.*, 2017; PAUL *et al.*, 2017). One of the key aspects of climate change is the rise in temperatures (FAHAD *et al.*, 2017; KARIMI; KARAMI; KESHAVARZ, 2018; STEINER *et al.*, 2018), which is expected to be 1.5-2°C by the end of 21st century (PACHAURI *et al.*, 2014). Heat or temperature rise is a significant uncontrollable factor affecting potato growth and ultimately reducing potato yield (MUTHONI; KABIRA, 2015; RAYMUNDO *et al.*, 2017; SINGH *et al.*, 2015; TRAPERO-MOZOS *et al.*, 2018). Tuberization is a complex mechanism which involves many physiological, molecular, metabolic and hormonal processes with the interaction to environmental factors (DUTT *et al.*, 2017; HANCOCK *et al.*, 2014; LEHRETZ *et al.*, 2019). Optimum temperature between 14 and 22°C is considered the best range for tuberization and gaining maximum yield (AIEN *et al.*, 2011; MUTHONI; KABIRA, 2015; SINGH *et al.*, 2016; TRAPERO-MOZOS *et al.*, 2018). Reduction in tuber formation occurs above 22ºC and there may not be any tuberization at 25ºC and above, whereas potato plants can withstand up to 32°C without significant loss in the total biomass production (SINGH *et al.*, 2016; TRAPERO-MOZOS *et al.*, 2018).

There is a huge amount of genetic diversity present in potato wild relatives which can be used through traditional approaches in plant breeding to gain sources of resilience to pests and adaptation to abiotic stresses (BASHIR; NICOLAO; HEIDEN, 2021). For example, F1 interspecific hybrids of *S. phureja* and *S. chacoense*, *S. berthaultii*, and *S. microdontum* crossed with dihaploid *S. tuberosum* subsp. *andigenum* produced diploid hybrids later crossed with *S. tuberosum* resulting in tetraploid hybrids resistant to heat stress. Other wild potatoes such as *S. boliviense*, *S. chacoense*, *S. iopetalum*, *S. kurtzianum*, *S. polyadenium*, and *S. raphanifolium* were somewhat heat resistant too (MACHIDA-HIRANO, 2015).

The success of a plant breeding program depends on the capacity to deliver genotypes that guarantee high performance in terms of efficiency and/or quality across a range of environmental conditions. Genotype by environment interaction (G×E) is the result of a differential response of genotypes across environments. Analyses and estimations of G×E have the potential to generate information on the characteristics of genotypes, identifying the superior ones for specific environmental conditions (Resende 2006). Wild potatoes have wide adaptability and high efficiency potential, as determined by G×E (FUMIA *et al.*, 2022). The most desirable cultivar is the one combining high efficiency and stability (DE RESENDE,

2002).

Prediction of the genetic values of genotypes is the most important aspect in breeding program, which requires estimating variance components that are either known or accurately estimated. Thus, the optimal procedure for estimating the residual or restricted maximum likelihood (REML) variance components and the optimal procedure for predicting the best linear unbiased prediction (BLUP) of genetic values are both associated with a mixed linear model. BLUP values show predictive accuracy when compared to other procedures, since the pedigree information is often included via the numerator relation matrix, which is often susceptible to analysis via a simple mixed model. The estimation of genetic values is mainly based on models with random effects. In studies on genetic breeding, consideration of treatment effects as random effects leads to greater predictive accuracy. This is relevant in genetic breeding programs and allows for genetic selection. Otherwise, the selection is phenotypic rather than genetic (MARCELO SORIANO VIANA *et al.*, 2012; VIANA *et al.*, 2011).

This eliminates variations and brings the response value closer to the genotypic response (PIEPHO, 1994; PIEPHO *et al.*, 2008). Some of the work on selection in advanced stages (close to obtaining a variety) based on BLUP methodologies has been carried out in species such as beans (DE CARVALHO; NETO; GERALDI, 2008), cassava (CEBALLOS *et al.*, 2016), corn (BERNARDO, 1996; OLIVOTO *et al.*, 2017), oil palm (PURBA *et al.*, 2001), sugarcane (BARBOSA *et al.*, 2014) and yams (BORGES *et al.*, 2010).

One of the main challenges of potato breeding is the expansion of cultivation to diverse climatic conditions since it requires considering the adaptability of the species to unfavorable environmental conditions and the maintenance of its production stability. However, no studies on wild potato species using missed model methodologies are found in the literature. These methodologies make it possible to predict the true genotypic values (F. NETO *et al.*, 2007; LIN; BINNS, 1988; PIEPHO *et al.*, 2008; TANG *et al.*, 2020). Development of heat-tolerant potato varieties through wild potato breeding is not only to adapt to climate change but also could create opportunities to grow potato in tropical regions throughout the world (GEORGE *et al.*, 2017). In this case, the present study aimed to rank the wild potatoes under two ranges of temperatures based on experimentation and by investigating adaptability and stability parameters through mixed modeling.

2.3. Material and methods

2.3.1. Plant material

Since 1986, Embrapa Temperate Agriculture, located in Pelotas, Rio Grande do Sul Sate, Brazil, has been developing activities aimed at rescuing, conserving, and using wild potato genetic resources that are geographically dispersed in southern Brazil. Potato germplasm has been evaluated for several horticultural traits over the years (Castro et al 2006). So, as a part of on-going research activities following research was conducted from 01/29/2019 to 04/23/2019 at Embrapa Temperate Agriculture, Pelotas, RS, Brazil (31° 40′ 34″ S 52° 26′ 28″ W) under controlled conditions. As part of germplasm conservation, continuous multiplication of wild genotypes already being done. So, on the basis of available multiplied germplasm we evaluate 21 potato and wild potato accessions (Table 1) from the Embrapa Potato Genebank which were collected from different regions of Brazil or introduced from abroad and are designated as identification codes BGB+number, Alelo Portal http://alelo.cenargen.embrapa.br/ (DEVA RODRIGUES, 2017) and BRA Genesys accession number https://www.genesys-pgr.org/ (LAWSON; BURTON; HUMPHRIES, 2018), further information on each accession can be accessed by clicking the accession number in the table and the commercial cultivar BRSIPR BEL, developed by Embrapa and Instituto de Desenvolvimento Rural do Paraná (IAPAR) (PEREIRA *et al.*, 2015).

The experiment follows a randomized complete block design (RCBD) with 2 factors, genotypes (G) and environment conditions designated as control (C) and stress (HS). 12 healthy tubers for each accession were selected and kept on phenolic sponge, for acclimatization from cold storage to sprouting in room temperature, being regularly irrigated with foliar solution for healthy growth. After 20 days, three most healthy and uniform sprouting tubers for each treatment (control & stress) were transferred to 3-liter plastic bags filled with organic substrate and NPK fertilizer as recommended for the potato crop in Brazil (SOCIEDADE BRASILEIRA DE CIÊNCIA DO SOLO, 2004). After 14 days of development, the plantlets were moved to the chambers under control and heat stress conditions (Table 2), according to a factorial experimental design, plants remained in growth chambers for 62 days and were 84 days old at harvest. Regular irrigation and pest scouting were performed daily.

Table 1: Germplasm from Embrapa Potato Genebank evaluated for genotypic response under heat stress

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

2.3.2. Environmental conditions

Two temperature ranges were applied in separate controlled chambers (Tabel 2). Chamber-1, which was designated as control, had a temperature range 14-27°C and chamber-2 for heat treatment, with temperature range 24-34°C. Both chambers had controlled photoperiod of 12 hours (7:00 at 19:00h) with light intensity 400 µmol m^{-2} s^{-1}.

Table 2: Growth chambers control and heat stress environmental conditions, with controlled, temperature, and humidity

Chamber-1: Control			Chamber 2: Heat Stress		
Time	**Temperature ºC**	**Humidity %**	**Time**	**Temperature ºC**	**Humidity %**
00:00-04:00	19	65	23:00-01:00	27	65
04:00-06:00	15	65	01:00-04:00	26	65
06:00-09:00	14	65	04:00-06:00	25	65
09:00-10:00	16	50	06:00-09:00	24	50
10:00-11:00	19	50	09:00-11:00	27	50
11:00-12:00	23	50	11:00-12:00	30	50
12:00-14:00	25	50	12:00-14:00	31	50
14:00-18:00	27	50	14:00-18:00	34	50
18:00-21:00	26	50	18:00-21:00	31	50
21:00-00:00	23	65	21:00-23:00	28	65

2.3.3. Agronomic Traits

All the agronomic traits were measured when plants were harvested 84 days after planting. Tuber yield traits evaluated were Fresh Shoot Weight (FSW), Dry Shoot Weight (DSW), Number of Smaller Tubers (NST), Number of Bigger Tubers (NBT), Number of Total Tubers (NTT), Weight of Smaller Tubers (WST), Weight of Bigger Tubers (WBT), Weight of Total

Tubers (WTT), and Dry Matter Content (DMC). All traits were measured following the standard protocols provided by the International Potato Center (CIP) (BONIERBALE, 2007). Bigger tubers were the ones larger than 2×2×2 cm and smaller tubers the ones narrower than 2×2×2 cm.

2.3.4. Statistical analysis

Estimates of the variance components and predictions of the genetic values were made using the REML/ BLUP (Restricted Maximum Likelihood/Best Linear Unbiased Predicted) procedure. The simultaneous selection for yield, stability and adaptability of genotypes was based on the harmonic mean of the relative performance of the predicted genetic values (HMRPGV). All these analyses were done using the model below (MENDES *et al.*, 2012).

The following statistical model was adopted for the evaluation of genotypes in the randomized block design with one observation per plot and in various environments or locations:

y = Xb + Zg + Wc + e, where y, b, g, c, e = data vectors of fixed effects (block means), of (random) genotypic effects of genotypes, of (random) effects of the genotype × environment interaction, and of random errors, respectively. X, Z and W = matrixes of incidence of b, g and c, respectively.

Distributions and structures of means and variances

$$E\begin{bmatrix} y \\ g \\ ge \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \\ 0 \end{bmatrix}; Var\begin{bmatrix} g \\ ge \\ e \end{bmatrix} = \begin{bmatrix} I\sigma_g^2 & 0 & 0 \\ 0 & I\sigma_{ge}^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

Mixed model equations:

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + 1\lambda_1 & Z'W \\ W'X & W'Z & W'W + 1\lambda_2 \end{bmatrix}\begin{bmatrix} \hat{b} \\ \hat{g} \\ \hat{g}e \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix}$$, here

$\lambda_1 = \frac{\sigma_e^2}{\sigma_g^2} = \frac{1-h_g^2-c_{ge}^2}{h_g^2}$; $\lambda_2 = \frac{\sigma_e^2}{\sigma_{ge}^2} = \frac{1-h_g^2-c_{ge}^2}{c_{ge}^2}$

$h_g^2 = \frac{\sigma_g^2}{\sigma_g^2+\sigma_{g_e}^2+\sigma_e^2}$ individual broad-sense heritability within a block;

$c_{g_e}^2 = \frac{\sigma_{g_e}^2}{\sigma_g^2+\sigma_{g_e}^2+\sigma_e^2}$ coefficient of determination of the effects of genotype × environment interaction;

σ^2_g: genotypic variance among genotypes;

σ^2_c: variance of the genotype × environment interaction;

σ^2_e: residual variance among plots;

Phenotype of the genotypes is influenced by its genetic makeup, environmental factors, and genotypes interaction with the environments where they are grown. The individual phenotypic variance is a sum of the genotypic variance to the residual variance between plots and to the variance in the genotype × environment interaction.

The predicted genotypic values, together with the SE which is the square root of residual variance corresponds to the standard error (SE) estimate can be used to obtain confidence intervals of the predicted genotypic values through the expression $(\mu + g) \pm t.SE$, where t = 1.96 is the tabulated value of the t distribution of Student. These results are referred to the lower and upper limits of the confidence interval, respectively.

$r_{gloc} = \frac{\sigma_e^2}{\sigma_g^2 + \sigma_{ge}^2} = \frac{h_g^2}{h_g^2 + c_{ge}^2}$ genotypic correlation across the environments. Estimators of components of variance by REML via algorithm EM

$\hat{\sigma}_e^2 = [y'y \quad - \quad \hat{g}'Z'y \quad - \quad \hat{c}'W'y]/[N \quad - \quad r(x)]$,

$\hat{\sigma}_g^2 = [\hat{g}'\hat{g} \quad + \quad \hat{\sigma}_e^2 tr\ c^{22}]/q$, and

$\hat{\sigma}_g^2 = [g\hat{e}'\hat{g}e \quad + \quad \hat{\sigma}_e^2 tr\ c^{33}]/s$,

Where C22 and C33 were derived from

$C^{-1} = \begin{bmatrix} c_{11} & c_{12} & c_{13} \\ c_{21} & c_{22} & c_{23} \\ c_{31} & c_{32} & c_{33} \end{bmatrix}^{-1} = \begin{bmatrix} c_{11} & c_{12} & c_{13} \\ c_{21} & c_{22} & c_{23} \\ c_{31} & c_{32} & c_{33} \end{bmatrix}$,

C = matrix of the coefficients of the mixed model equations;

tr = trace of a matrix operator;

r(x) = rank of matrix X;

$\hat{r}_{gg}^2 = \sqrt{\hat{h}_g^2}$ corresponds to genotype selection accuracy.

N,q,s = total number of data, number of genotypes and number of genotype × environment combinations, respectively. The genetic gain was calculated as the average of the breeding values of the selected individuals. The selection was performed using the breeding values of the best genotypes for each trait. The genotypic values of each clone were obtained by adding each genotypic effect to the overall mean. The genetic gain was equal to the average of the vectors of the predicted genetic effects for the selected accession. The overall mean plus the genetic gain resulted in the improved average population. The relative performance of each accession was determined using the relationship between the average of the improved population for each accession and the genetic value of the better accession, depending on the direction of selection.

In this model, the interaction-free predicted genotypic values considering all locations are given

by u + g, where u is the mean of all locations. For each location j, the genotypic values are predicted by µj+g+ge, where µj is the mean of location j.

The joint selection for yield, stability and adaptability of the plant material was based on a parameter named harmonic mean of the relative performance of the predicted genetic values (HMRPGV), as described by (Resende 2004). Results of the HMRPGV are similar to those obtained by the methods described by (LIN; BINNS, 1988) and (ANNICCHIARICO, 1992). All analyses were performed on software SELEGEN-REML/BLUP, model 54 (Resende 2002).

2.4. Results

2.4.1. REML Variance Components

The estimation of genetic parameter of all studied traits are shown in Table 3. Highest individual broad sense heritability was observed for FSW and DSW with value of 0.59±0.19 both and lowest by WST at 0.24±0.12. The average heritability of genotypes $\left(\hat{h}^2_{AG}\right)$ value obtained for all trait's ranges from 0.57 to 0.89. NBT contains the lowest value for genotypic, phenotypic, and residual variances while the highest value was observed for the FSW as 2388.70, 4018.75 and 1083.41, respectively, except for residual variance which is showed by WTT by 839.11. Highest correlation across environment and accuracy in genotypic selection was observed for the DMC. Residual variance ($\hat{\sigma}^2_e$) shows maximum effect to the total phenotypic variance ($\hat{\sigma}^2_f$) for WST (61.13%) and for other traits 22.96% to 42.29% (Table 3). The interaction ($\hat{\sigma}^2_{int}$) in the current study corresponds for low 0.32% (DMC) and highest for 30.83% (WBT) to the phenotypic variance. The value of the interaction variance ($\hat{\sigma}^2_{int}$) can be used to access the expression of genotype depend upon its genetic adaptability and stability degree in particular environment. Thus, it also allows estimating a low to high correlation across environments ($\hat{r}_{gloc}$), with minimum value observed for WBT (0.58) and maximum for the DMC (0.99). Meanwhile, all other traits show high genotypic correlation across both environmental conditions. Highest genotypic accuracy across environments was observed for DMC (94%), followed by DSW, FSW, NBT, NTT, WTT, NST, WBT and least for WST, classified according to (RESENDE; DUARTE, 2007). Obtained values of CV_e ranges from 19.82% to 103.55% with lowest for DSW and highest for the WST. In case of CV_g ranges low for DSW and high for NST, 25.70% to 78.23% respectively, which gives a strong basis of the obtained results.

Table 3: REML (Restricted Maximum Likelihood) variance component estimates in potatoes under control temperature and heat stress conditions. Abbreviatures: FST (Fresh Shoot Weight), DSW (Dry Shoot Weight), NBS (Number of Bigger Tubers), NST (Number of Smaller Tubers), NTT (Number of Total Tubers), WBT (Weight of Bigger Tubers), WST (Weight of Smaller Tubers), WTT (Weight of Total Tubers), DMC (Dry Matter Content).

TRAITS		FSW (g)	DSW (g)	NBT	NST	NTT	WBT (g)	WST (g)	WTT (g)	DMC (%)
ANOVA	Genotype	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
	Treatment	0.000***	0.000***	0.000***	0.001**	0.000***	0.000***	0.002**	0.000***	0.007**
	Genotype × Treatment	0.001**	0.179^{ns}	0.000***	0.000***	0.000***	0.000***	0.034*	0.000***	0.853^{ns}
$\hat{h}^2_g$: *Broad sense heritability*		0.59±0.19	0.59±0.19	0.54±0.19	0.42±0.16	0.55±0.19	0.43±0.17	0.24±0.12	0.48±0.17	0.57±0.19
$\hat{h}^2_{AG}$: *Heritability of genotype average*		0.84	0.87	0.80	0.71	0.78	0.68	0.57	0.72	0.89
$\hat{\sigma}^2_g$: genotypic variance		2388.70	24.51	13.44	31.75	82.46	1032.45	61.95	1392.48	57.55
$\hat{\sigma}^2_f$: *phenotypic variance*		4018.75	41.32	25.07	75.65	151.25	2401.12	259.55	2920.61	100.27
$\hat{\sigma}^2_e$: *residual variance*		1083.41	14.58	7.20	26.35	34.28	628.51	158.66	689.02	42.41
$\hat{\sigma}^2_{int}$: $G \times E$ *variance*		546.63	2.23	4.44	17.56	34.51	740.15	38.94	839.11	0.32
$\hat{r}_{gloc}$: *correlation across enviroments*		0.81	0.92	0.75	0.64	0.70	0.58	0.61	0.62	0.99
$\hat{r}_{ac}$: accuracy in genotypic selection		0.92	0.93	0.89	0.84	0.88	0.83	0.76	0.85	0.94
General mean		118.21	19.26	5.07	7.20	12.28	50.56	12.16	62.73	20.74
CV_g%: coefficient of genetic variation		41.35	25.70	72.25	78.23	73.97	63.55	64.70	59.49	36.57
CV_e%: coefficient of exp. variation		27.85	19.82	52.88	71.27	47.69	49.58	103.55	41.85	31.39

2.4.2. Genotypic selection under Heat Stress conditions

This study focused on the selection of best genotypes under joint analysis of two different temperature ranges and specifically under heat stress (HS) conditions. Selection of potato genotypes based on genotypic response was further verified by using 5 different strategies named as selection under heat stress, selection under joint analysis, Stability of genotypic values by the harmonic mean of genotypic values (HMGV), adaptability of genotypic values by the relative performance of genotypic values (RPGV), stability and adaptability of genotypic values by the harmonic of the relative performance of genotypic values (HMRPGV), respectively shown in Fig 1 to 9 and Supplementary Table 1S.

Fig 1 to Fig 9 indicate the genotypic values and genetic gain for studied germplasm observed for heat stress (HS) annotated as “A” and joint analysis (JA) annotated as “B" for all traits FSW, DSW, NBT, NST, NTT, WBT, WST, WTT and DMC respectively. Results showed that under stress conditions, BGB467 attained the highest genotypic value along with high genetic gain for vegetative traits FSW and DSW while the lowest was observed for BGB086. BGB113 was ranked 1st with high genotypic value and genetic gain for NBT, NTT, WST and WTT. Traits like NST, WBT and DMC highest predicted values were observed for BGB088, BGB091 and BGB451 respectively. Observed lowest performer for bigger tuber traits was BGB045 and for smaller tubers was BGB009. In Joint analysis ranking, order was same as observed under stress for first and last ranked genotype except for NST and WTT, where ranking starts with

BGB113 and BGB088, respectively.

For trait FSW (Fig 1), 9 genotypes (BGB467, BGB098, BGB451, BGB444, BGB113, BGB109, BGB101, BGB103 and BGB045) showed higher genotypic values (μ+g+ge) under stress order for joint analysis was BGB467, BGB451, BGB098, BGB444, BGB103, BGB109, BGB045, BGB101, BGB472 and BGB113 (μ+g) than the average mean performance of the wild genotypes and higher than the commercial cultivar BEL. The genetic gain resulting from the used genotypes for FSW ranged from 0 to 122.50% and 0 to 128.39% in both heat stress and joint analysis, respectively. Same genotypes have similar results through other methods which are genotypic stability by the method of harmonic mean of the genotypic values (HMGV), genotypic adaptability by the method of relative performance of predicted genotypic values (RPGV) across environments, a simultaneous measure of productivity, stability, and adaptability by the method of harmonic mean of the relative performance of the genotypic values (HMRPGV) and with similar order (Table 1S).

Despite the focus of this study is to evaluate potato wild relative accessions, it is remarkable that the *S. tuberosum* dihaploid BGB088 was found among top six of genotypic values for most of the traits (NBT, NST, NTT, WBT, WST, WTT) along with the diploid *S. chacoense* BGB113. These two genotypes show better performance than the general mean of all genotypes evaluated. Among all the methodologies, genotypic values under HS, joint analysis, HMGV, RPGV and HRPGV have similar order predicts the importance of these genotypes. Moreover, the remaining genotypes among top 6 selected in all methodologies belongs to the *S. commersonii* (BGB451) and *S. chacoense* group (BGB102, BGB103) the order of the top genotypes were similar of that specific trait (Fig 2-9; Table 1S).

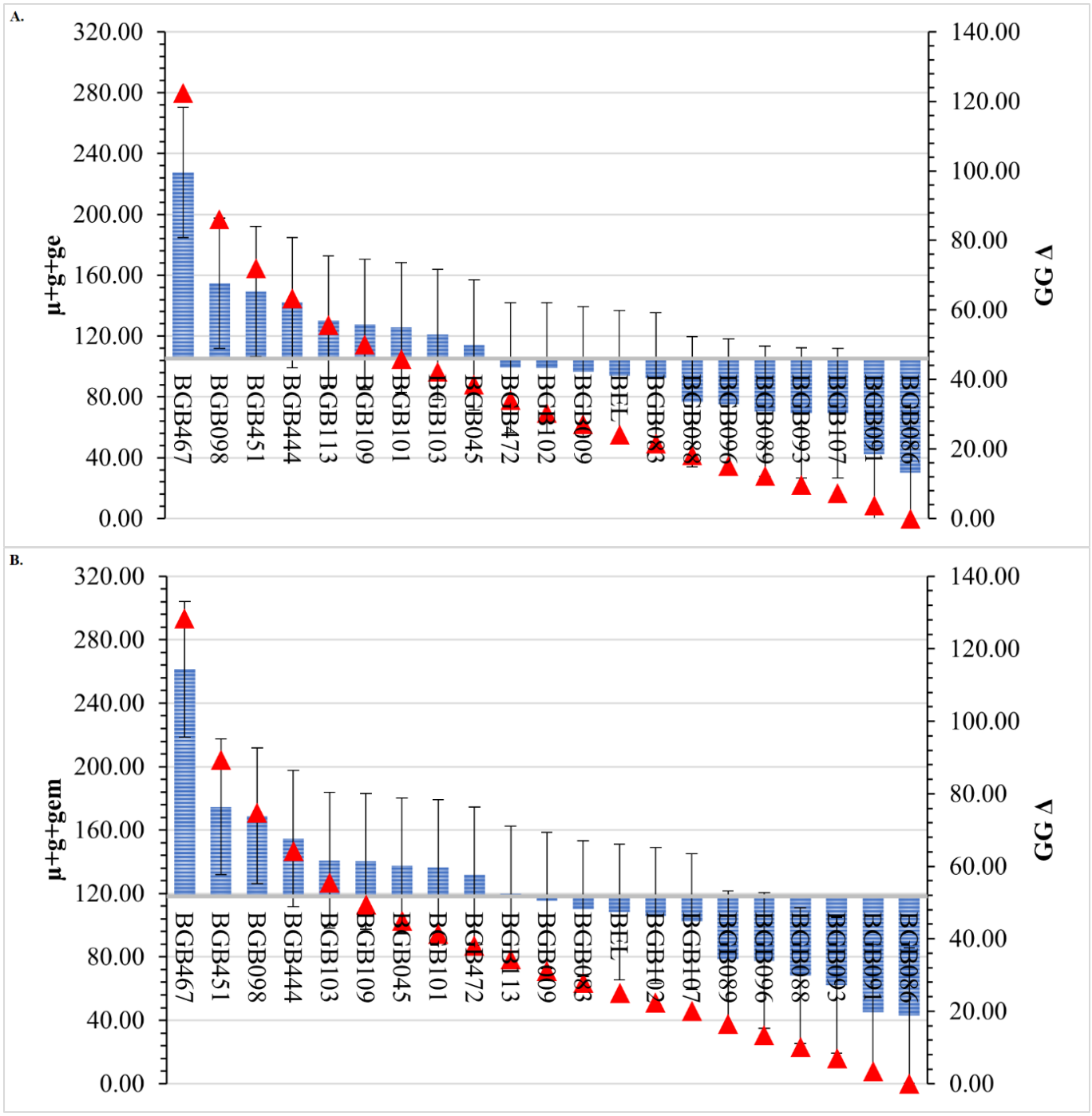

Figure 2: A- Genotypic values (μ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (μ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Fresh Shoot Weight - FSW (g).

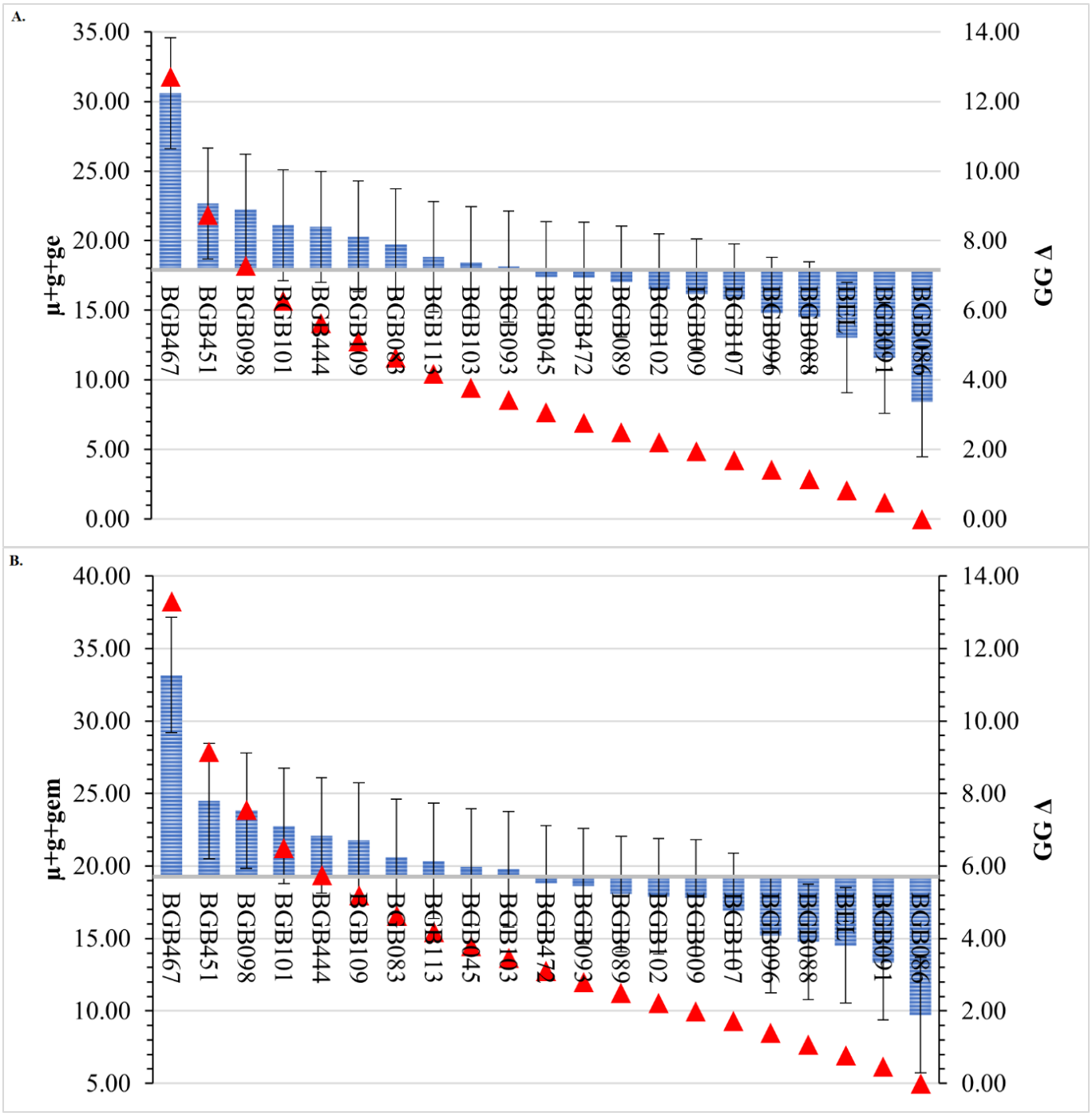

Figure 3: A- Genotypic values (µ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (µ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Dry Shoot Weight - DSW (g).

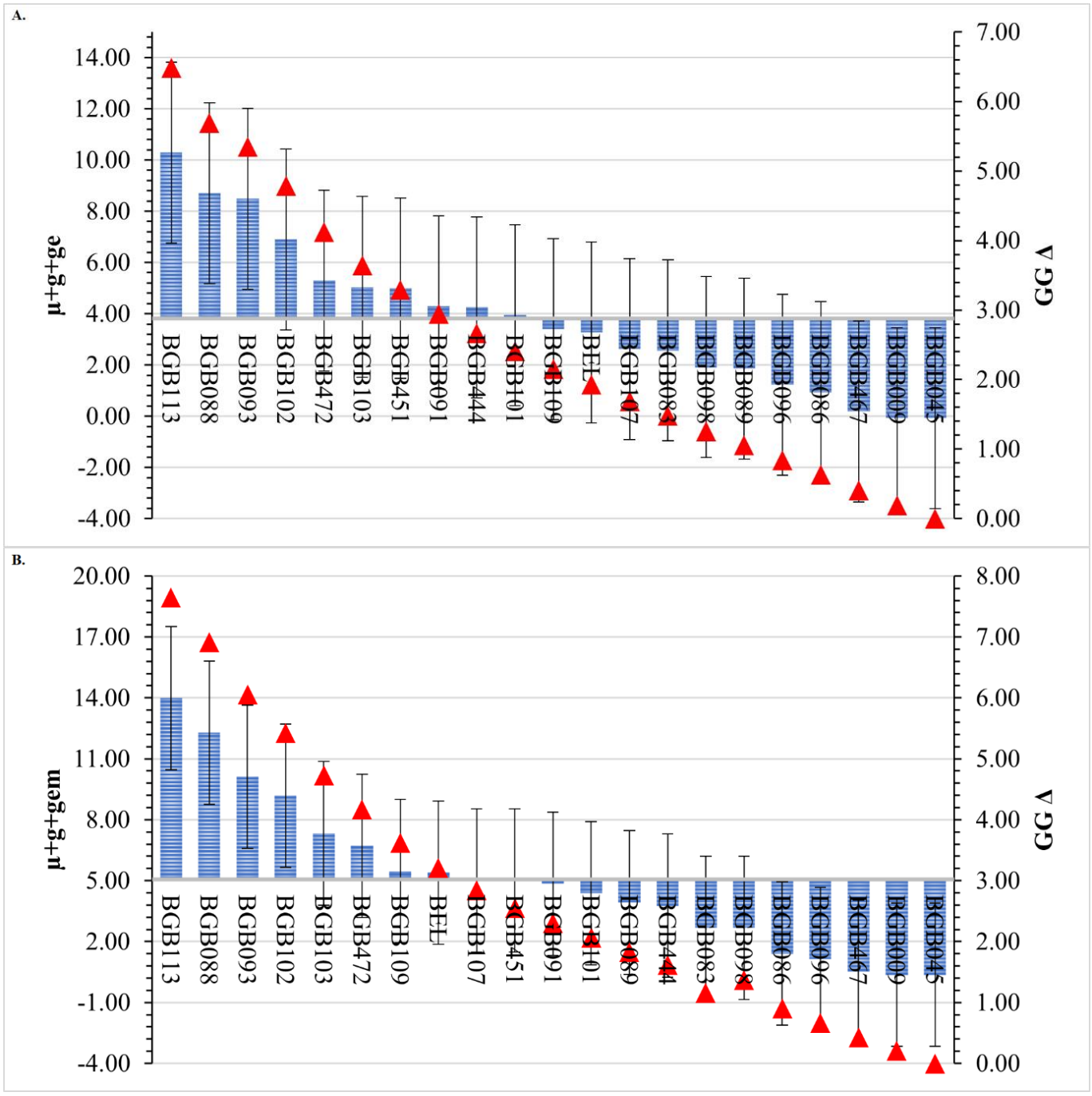

Figure 4: A- Genotypic values (μ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (μ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Number of Bigger Tubers - NBT.

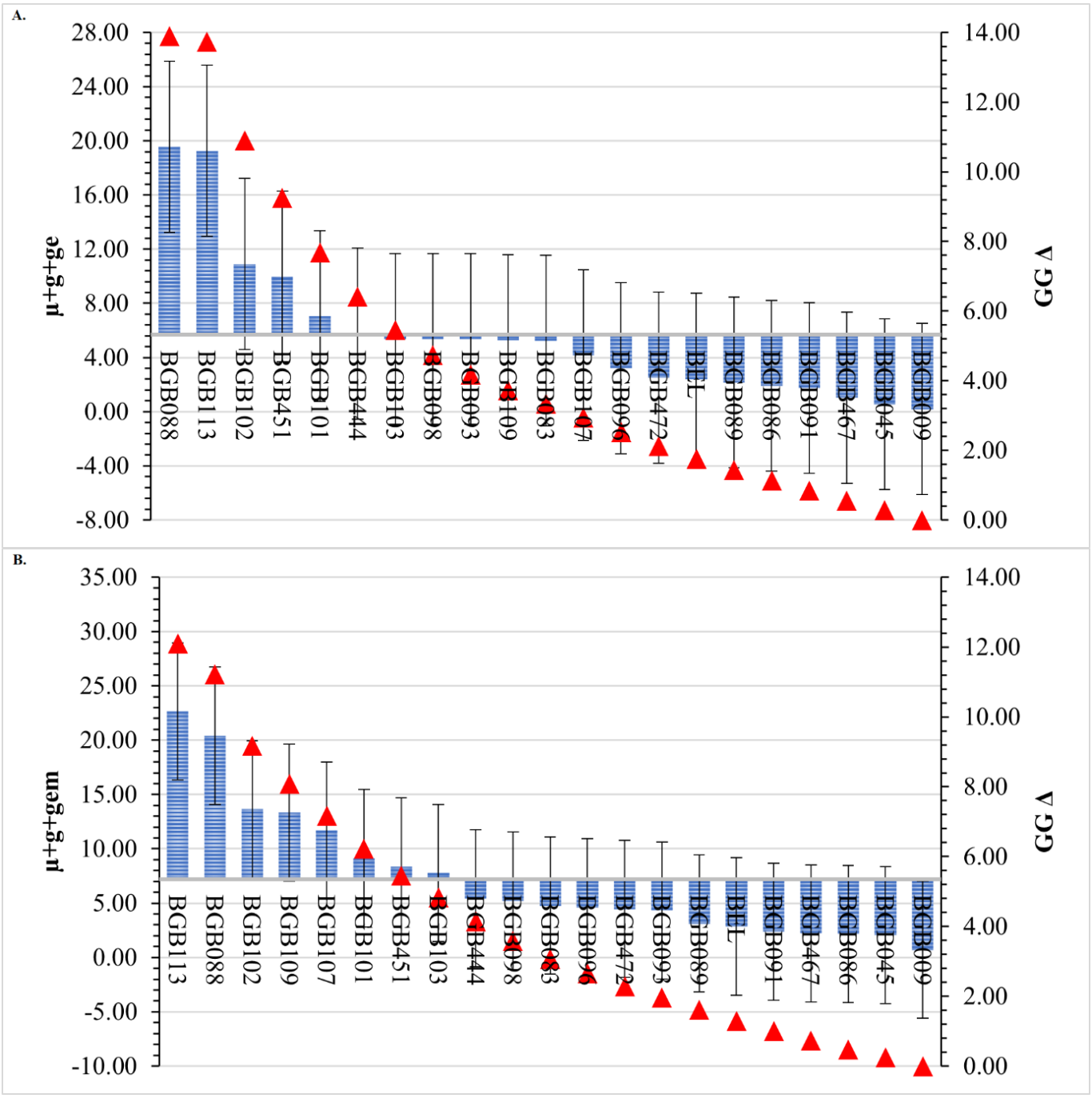

Figure 5: A- Genotypic values (µ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (µ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Number of Smaller Tubers - NST.

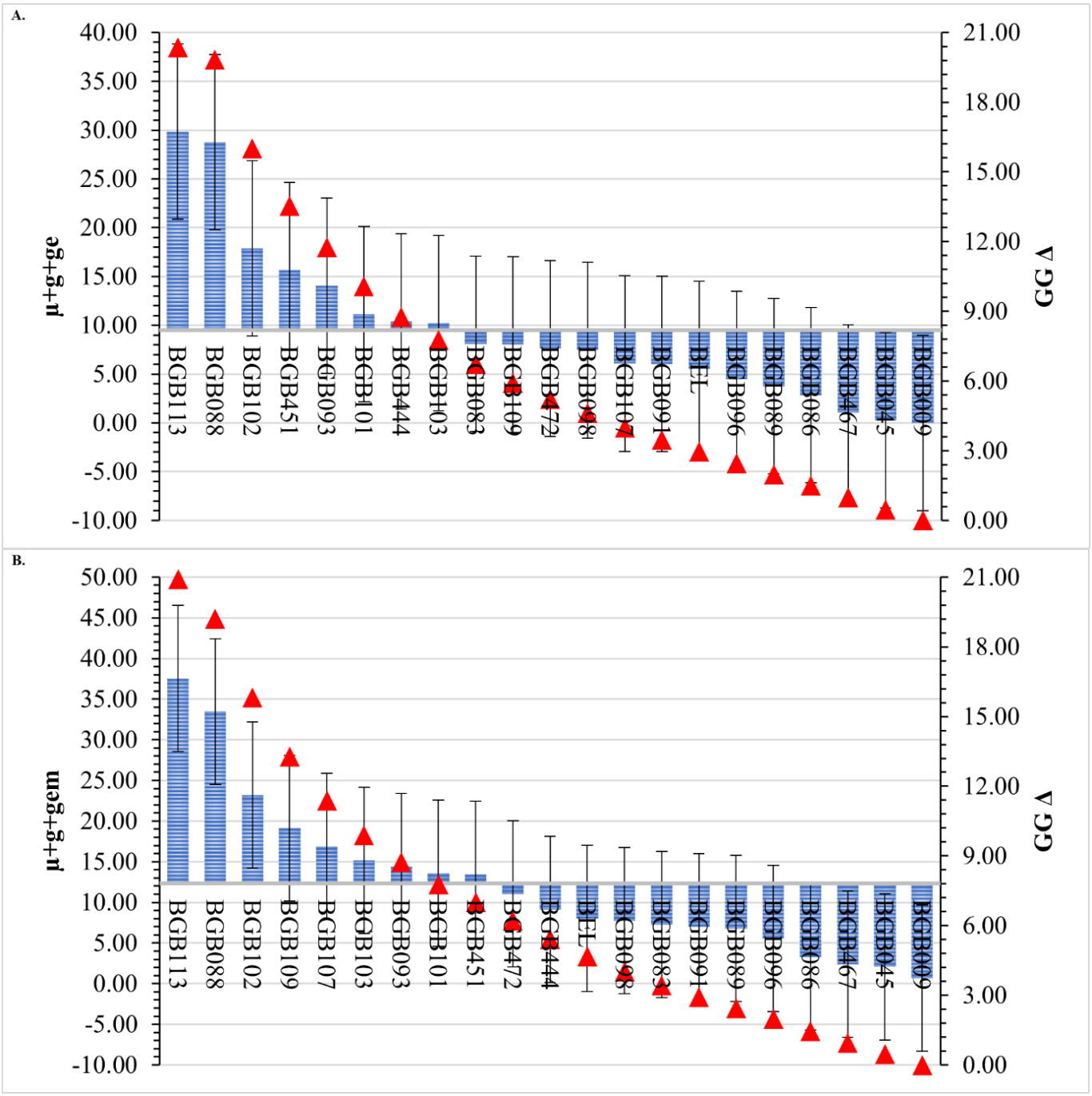

Figure 6: A- Genotypic values (μ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (μ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Number of Total Tubers - NTT.

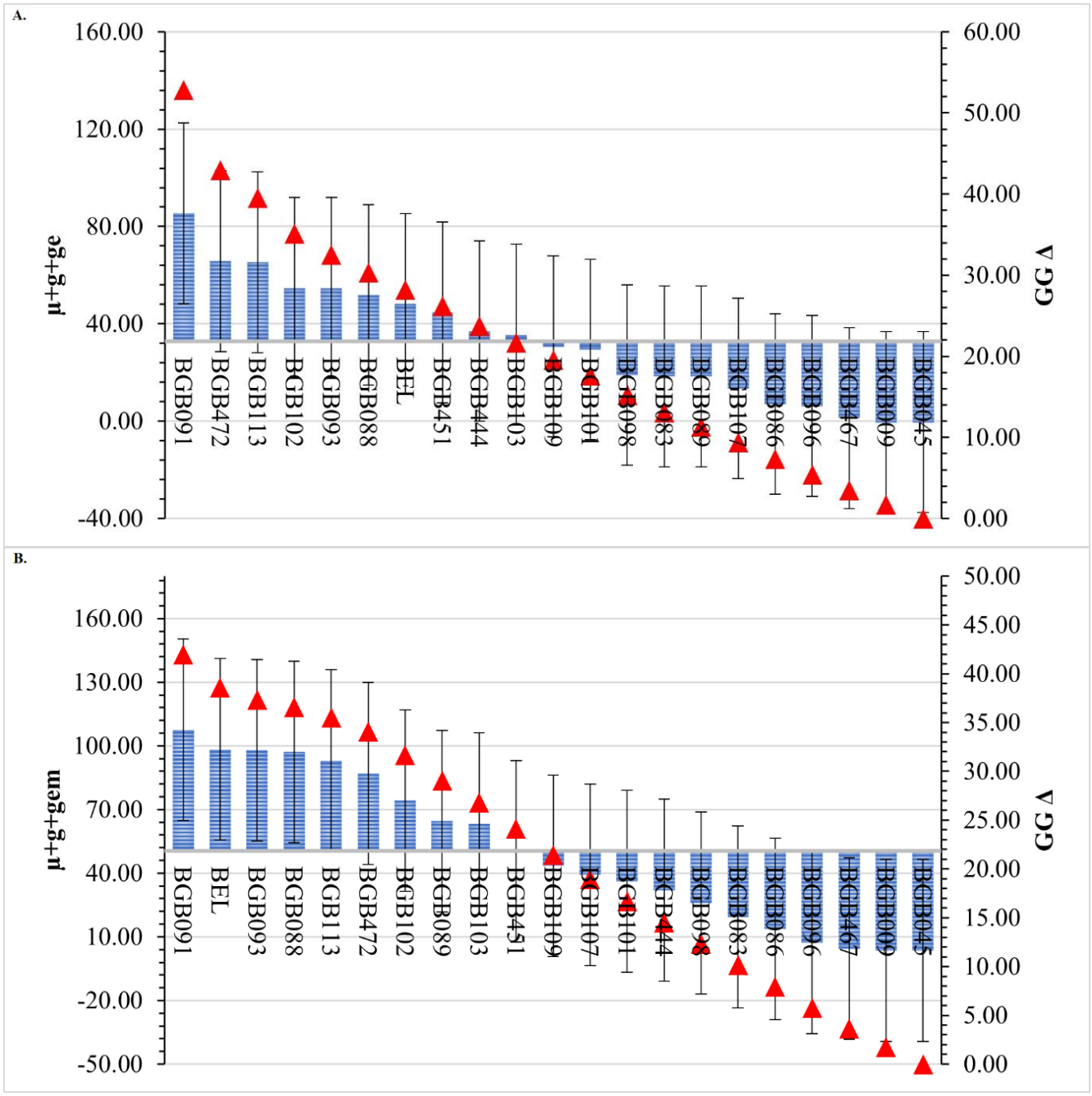

Figure 7: A- Genotypic values (µ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (µ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Weight of Bigger Tuber - WBT (g).

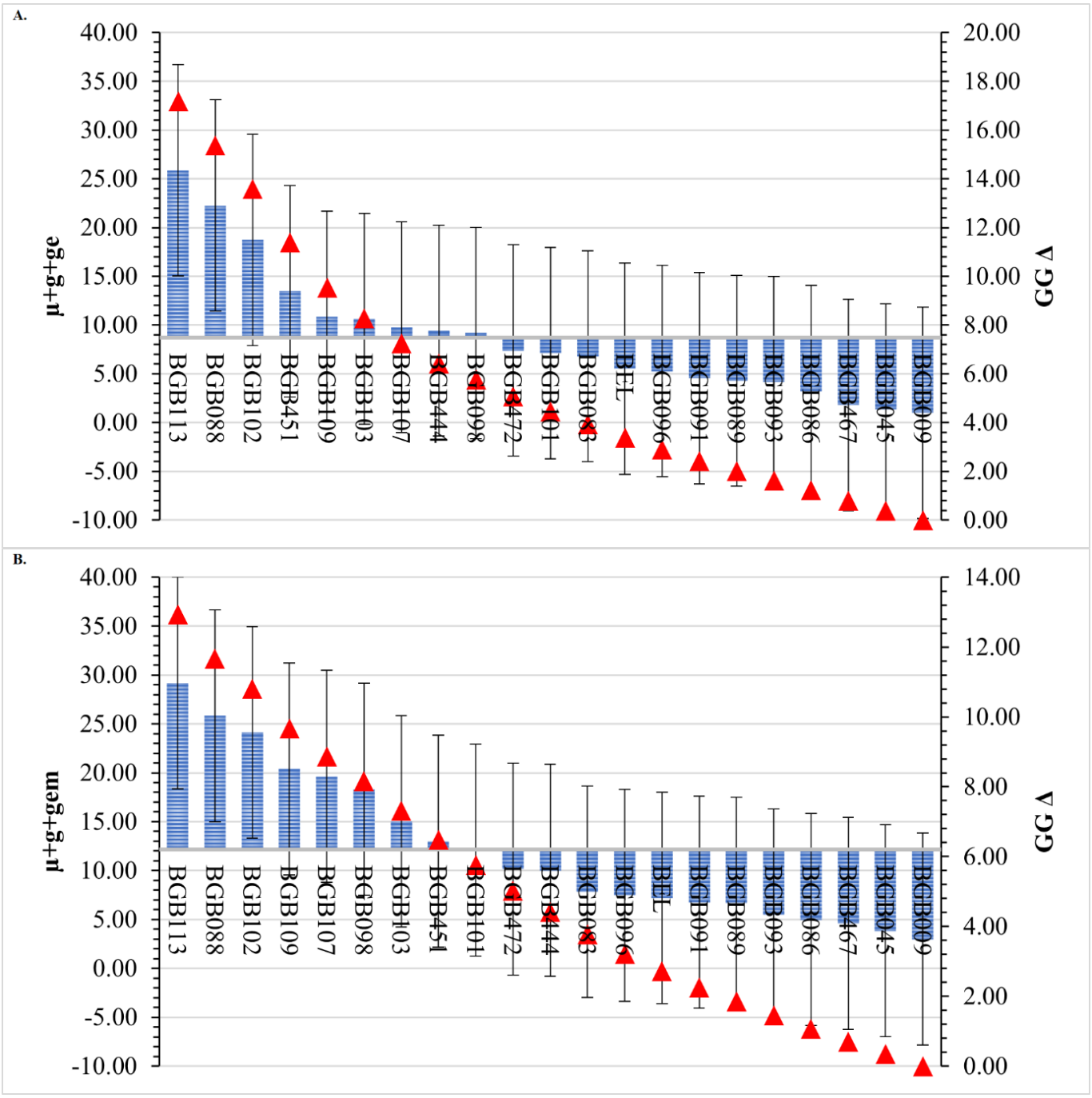

Figure 8: A- Genotypic values (μ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (μ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Weight of Smaller Tubers - WST (g).

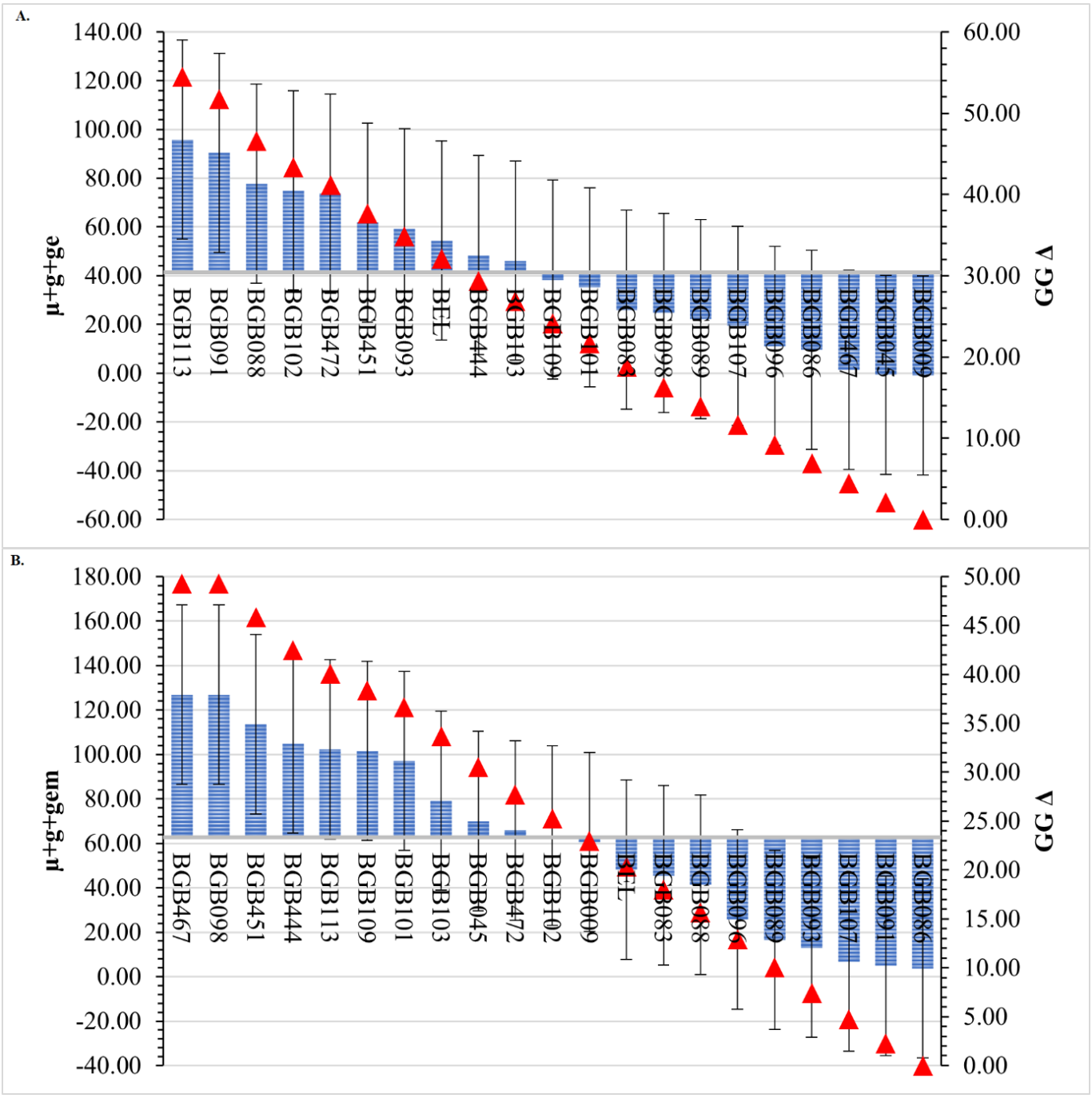

Figure 9: A- Genotypic values (μ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (μ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Weight of Total Tubers - WTT (g).

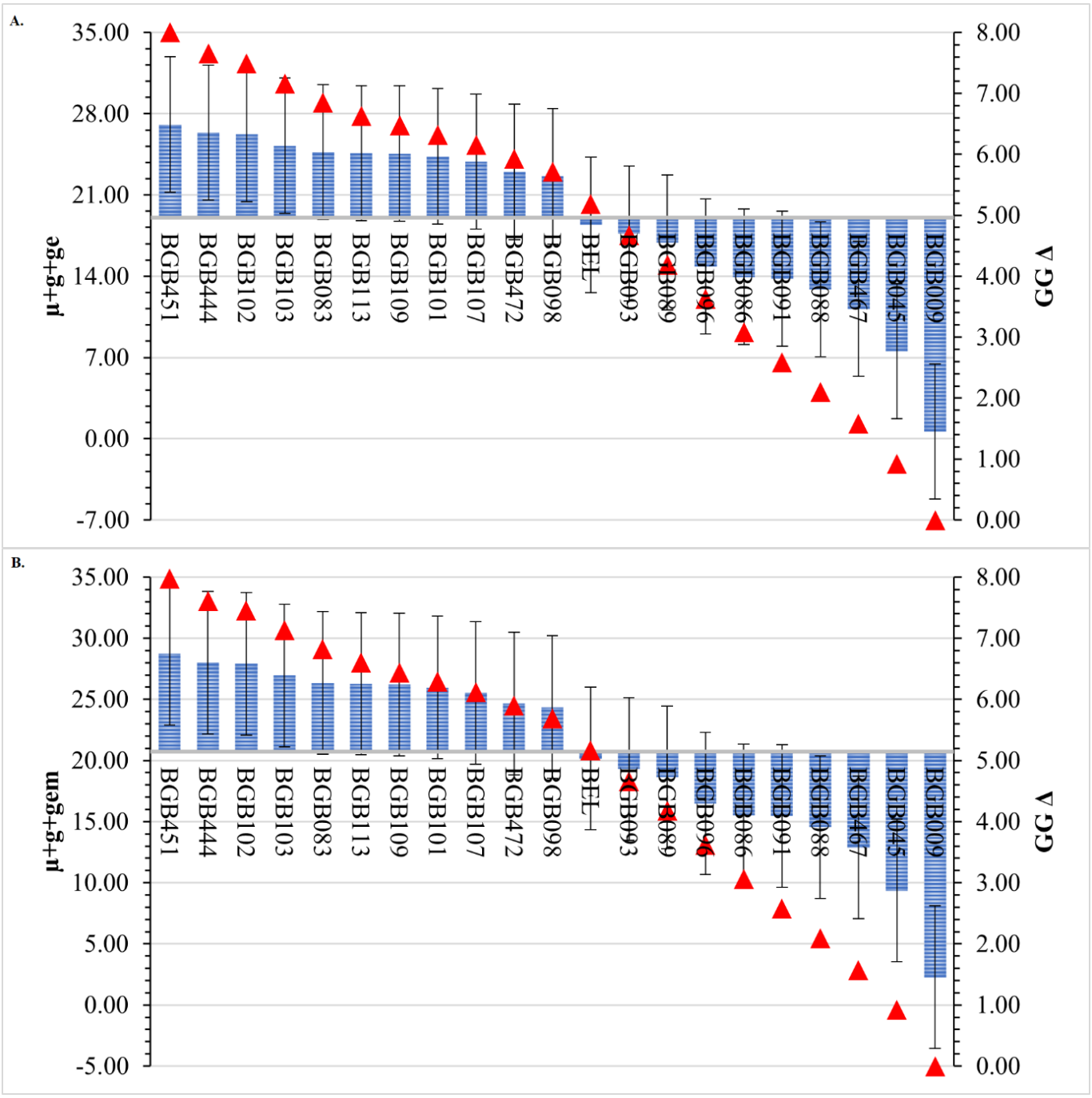

Figure 10: A- Genotypic values (µ+g+ge) and genetic gain (GG) of potato genotypes under Heat Stress (HS). B- Genotypic values (µ+g+ge) and genetic gain (GG) of Joint Analysis (JA) for trait Dry Matter Content – DMC (%).

Mixed model methodologies and selection of wild genotypes by ranking according to higher genotypic values and among the 6 selected genotypes genetic gain was significant for all traits under heat stress conditions. This selection was based on genotypes with high mean and genetic variability. For almost all traits, BGB113 *S. chacoense* and BGB088 *S. tuberosum* dihaploid showed considerable genetic gain. While the ranges of genetic gain observed under HS for FSW, DSW, NBT, NST, NTT, WBT, WST, WTT and DMC were 0-122.50%, 0-12.71%, 0-6.49%, 0-13.89%, 0-20.37%, 0-52.80%, 0-17.16%, 0-54.44% and 0-7.99% respectively.

The exact mean superiority of the highly responsive wild potato accessions can be estimated through relative performance of genotypic values (RPGV) and stability, adaptability of genotypic values by harmonic means of the relative performance of genotypic values (HMRPGV). These values allow a comparison of performance of genotype in a specific environment to the mean environment. Genotypes BGB088 and BGB113 which are among top 6 genotypes for most of the studied traits, the relative performance and stability, adaptability by harmonic mean of relative performance values for traits such as NST 2.94 times for BGB088 and 3.19 times for BGB113, respectively. For trait NBT 2.39 and 2.75 times, for NTT 2.78 and 3.07 times; for WBT 1.83 and 1.88 times; for WST 2.21 and 2.52 times; for WTT 1.98, 2.09 times, respectively. In the case of the remaining traits, these two genotypes were not found in top 6. The selection of these 2 genotypes through the HMRPGV method, by using predicted genotypic values (HMRPGV*GM) resulted in new mean of the traits studied which value is slightly greater than the observed genotypic values for the joint analysis of the studied environments.

Moreover, among *S. chacoense* (potato secondary genepool) assessed in this study, the diploid BGB113 was the highest and the triploid BGB086 was determined to be the lowest in yield. In the case of *S. commersonii* (potato tertiary genepool), the diploids BGB009 and BGB045 were the lowest in yield. Among all tested dihaploid *S. tuberosum* from Embrapa Potato Genebank, BGB088 was the highest in yield under stress conditions Which showed that this genotype has more potential than the rest of the used dihaploid genotypes in current studies.

2.5. Discussion

Wild potato tuber related traits are highly influenced by environmental factors (QUIROZ *et al.*, 2018; RAYMUNDO *et al.*, 2018). Ticona-Benavente and Silva-Filho 2015 suggested BLUP/REML method base selection for tuber yield is more efficient in case of first clonal generation. Environmental influence on tuber yield related traits was also observed by (PACHECO *et al.*, 2020) and (SILVA *et al.*, 2018). Tuber yield related traits are

mathematically quantitative and affected by environmental interaction. Traits related to tuber yield are important to economic profitability. Factually, there is need to maintain a balance between number of tubers per plant and tuber yield (DA SILVA *et al.*, 2006) because higher tuber number will produce small tubers which are not market preference (DA SILVA *et al.*, 2012). As tuber yield is a quantitative trait, environmental influence is always significant, so accuracy of the selection of clones is given as adequate as (DA SILVA *et al.*, 2006). For most of the traits related to tuber yield in this study, coefficient of variance for phenotype, genotype and experiment are ranges from medium to high which is in accordance with the report by (BISOGNIN *et al.*, 2008; DA COSTA *et al.*, 2007; DA SILVA *et al.*, 2012, 2006; SEID; MOHAMMED; ABEBE, [*s. d.*]; SILVA *et al.*, 2018).

Individual broad sense heritability ($\hat{h}_g^2$) for all traits ranged from the lowest to moderate value (0.24-0.59) in the joint analysis, because of the genotype × environment interaction. Range of the recorded deviation (±0.12-±0.19) did not allow the estimated broad sense heritability value to zero which is favorable for the traits under study. The average heritability of genotypes $\left(\hat{h}_{AG}^2\right)$ is based on the averages of the blocks as the criteria for evaluation and /or selection (DE RESENDE, 2004). Haynes et al. 1989 studied the heritability of diploid potatoes under high temperature growing conditions and observed the variability of dry matter content by specific gravity. The study concluded unbiased estimate of heritability was moderate to high which confirms the results of this study. Therefore, in view of obtained moderate to high observed values which indicates that the selection of superior wild genotypes can be based on predicted genotypic values.

The coefficient of genetic variation provide information about the portion of genetic variance extracted from the phenotypic variance and in this study of wild potatoes we obtained a range of low CV_g for DSW and highest for the NST. This range is an indication of genetic variation for the observed traits (DE PELEGRIN *et al.*, 2017). de RESENDE 2004 stated that higher values of coefficient of genotypic variation permits genetic gains in the selection of wild genotypes. For selection-based breeding programs calculation of CV_e and CV_g parameters is most relevant because it has direct effect on ($\hat{r}_{ac}$) selective accuracy of wild genotypes (RESENDE; DUARTE, 2007). Genotypic accuracy classified high for all traits, which shows the reliability of selection of wild potato genotypes by all measured traits considered in this study.

The assent of genotypic correlation is important to estimate because it presents the degree of reliability of selecting the best genotypes in our concerning environmental condition. As, the

higher magnitudes of correlation among environment indicates that G×E interaction for these traits expressed simple effects, in other words, although there was differentiated behavior, the genotypes classification was not substantially altered in function of the different tested environments. Higher levels of environmental effects imposed by interaction on these yield related traits justifies that due to their genetic nature they may be controlled by a large number of genes, differentially interacting with the environment which brings modification to phenotypic expression of traits (PUPIN *et al.*, 2015).

The data justifies the ranking of genotypes on the base of genotypic values obtained from BLUP, through methodologies of Stability (HMGV), adaptability (RPGV and RPGV*GM), and stability, adaptability of genetic values (HMRPGV and HMRPGV*GM) of genotypes. More than 80% of the ranking by genotypic values (Fig-1 to Fig-9) were in concordance with those methodologies. Because these methodologies showed the same ranking of genotypes as shown on the base of predicted genotypic values.

According to (FARIAS NETO *et al.*, 2013), increase or decrease yield depend according to genotypic performance associated to stability (HMGV), adaptability (RPGV), and both simultaneously (HMRPGV) for all under observed temperature ranges. If there is a total agreement between high performing genotypes based on HMGV, RPGV, HMRPGV, and average yield, these results show that secure predictions about genetic values can be made based on a single standard contemplating yield, stability, and adaptability (VERARDI *et al.*, 2009). The HMRPGV method selects genotypes based on their adaptability and stability, which is important to direct controlled crossings in evaluation phases of genetic breeding programs and to recommend superior genotypes for commercial use. Generally, a univariate model of repeatability, considering all locations simultaneously, is suitable for selection, focusing on the average yield in all locations. In the study by (STURION; DE RESENDE; RESENDE, 2005), a complete model was used to recommend specific genotypes for each location, selection of stable genotypes, selection of more adaptable genotypes to environmental stresses, and selection bearing in mind all aspects simultaneously. Hence, in the current study, the top six genotypes maintain their ordering for stability, adaptability, and both simultaneously. So selection of these genotypes for breeding program is worth searching which is in accordance with the results reported by (BASTOS *et al.*, 2007; CARVALHO *et al.*, 2017; DE PELEGRIN *et al.*, 2017; FARIAS NETO *et al.*, 2013; GONÇALVES *et al.*, 2014; OLIVEIRA *et al.*, 2020; SOUSA *et al.*, 2019; SOUZA *et al.*, 2018).

The value of germplasm is determined by its genetic diversity, accessibility, and usefulness. In this sense, potato emerge among all other crops (BAMBERG; DEL RIO, 2005). Wild potato

species from secondary genepool (MACHIDA-HIRANO, 2015) such as *S. acaule, S. ajanhuiri, S. boliviense, S. brevicaule, S. bulbocastanum, S. candolleanum, S. chacoense, S. chomatophilum, S. colombianum, S. curtilobum, S. demissum, S. infundibuliforme, S. iopetalum, S. jamesii, S. juzepczuckii, S. kurtzianum, S. medians, S. okadae, S. paucissectum, S. pinnatisectum, S. polyadenium, S. raphanifolium, S. sogarandinum, S. stoloniferum* and *S. vernei* have different levels of expression for tuber quality under abiotic stresses through diploid potato breeding strategy (ALI; JANSKY, 2015; BASHIR; NICOLAO; HEIDEN, 2021; BILSKI; NELSON; CONLON, 1988; HANNEMAN JR, 1996; LI, P H, 1977; MACHIDA-HIRANO, 2015; PINO *et al.*, 2013; ROSS; HUNNIUS, 1986; SABBAH; TAL, 1995; WATANABE *et al.*, 2011). Although genetic improvement for total tuber yield has not been understood (DOUCHES *et al.*, 1996). So, this study is a contribution towards understanding the genetic parameters which are important to study in diploid accessions to fill this gap. However, still there are missing strings that explains the hurdles to introgress the important tuber yield related traits to tetraploid cultivar for commercial use (BETHKE; HALTERMAN; JANSKY, 2017).

2.6. Conclusion

Overall, the wild relative genotypes were found superior in genotypic values for most of the traits. Based on the results, we highlight accession BGB113 diploid *S. chacoense* as the best performing one and BGB088 as the best performing dihaploid *S. tuberosum* genotype assessed. These two genotypes were also predicted by other methodologies such as HMGV, RPGV and HMRPGV, which shows they could potentially be used in breeding programs for diverse environments.

This study provides evidence that wild potato genotypes showed greater adaptive behavior, along with they were able to perform better than the tetraploid commercial cultivar for most of the traits measured under unfavorable potato crop conditions. Wild potatoes are low in tuber yield when compared to the domesticated commercial cultivars and could bear high levels of tuber glycoalkaloid contents. However, it is necessary to evaluate the tuber production of potato wild relatives under high temperature and to rank the best ones for genotypic selection based on adaptability, stability and yield due to presence of good predictability.

The recognition of potato germplasm able to set tubers under heat stress conditions is the first step towards identifying promising germplasm for introgression trials into the potato crop genepool. The advancement of introgressed pre-breeding lineages is aimed to the development of potato cultivars for expanding the potato crop area beyond the temperate zones and to breed

novel cultivars resilient to the challenges posed by climate change.

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2.8. References

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Supplementary Table 1: Stability of genotypic values by the harmonic mean of genotypic values (HMGV), adaptability of genotypic values by the relative performance of genotypic values (RPGV), stability and adaptability of genotypic values by the harmonic of the relative performance of genotypic values (HMRPGV), genotypic value capitalizing adaptability by the RPGV multiplied by the general mean of both control and stress conditions (RPGV*GM), and genotypic value penalized by instability and capitalized by adaptability by the HMRPGV multiplied by the general mean of both control and stress conditions (HMRPGV*GM) of potato accessions. Fresh Shoot Weight (g) (FSW), Dry Shoot Weight (g) (DSW), Number of Bigger Tubers (NBT), Number of Smaller Tubers (NST), Number of Total Tubers (NTT), Weight of Bigger Tubers (g) (WBT), Weight of Smaller Tubers (g) (WST), Weight of Total Tubers (g) (WTT), Dry Matter Content (%) (DMC).

FSW-Rank	Genotype	HMGV	Genotype	RPGV	RPGV*GM	Genotype	HMRPGV	HMRPGV*GM
1	BGB467	256.94	BGB467	2.21	260.77	BGB467	2.21	260.68
2	BGB451	170.85	BGB451	1.47	173.85	BGB451	1.47	173.65
3	BGB098	167.69	BGB098	1.43	169.40	BGB098	1.43	169.28
4	BGB444	153.56	BGB444	1.31	155.10	BGB444	1.31	154.96
5	BGB109	139.07	BGB109	1.19	140.53	BGB109	1.19	140.46
6	BGB103	137.99	BGB103	1.19	140.33	BGB103	1.19	140.19
7	BGB101	135.57	BGB101	1.16	136.92	BGB101	1.16	136.78
8	BGB045	133.42	BGB045	1.15	136.46	BGB045	1.15	135.98
9	BGB472	123.72	BGB472	1.10	129.74	BGB472	1.08	127.21
10	BGB113	118.66	BGB113	1.03	122.25	BGB113	0.99	117.53
11	BGB009	112.36	BGB009	0.97	114.81	BGB009	0.97	114.47
12	BGB083	107.31	BGB083	0.93	109.58	BGB083	0.92	109.28
13	BEL	106.41	BEL	0.91	108.09	BEL	0.91	108.03
14	BGB102	105.54	BGB102	0.90	106.54	BGB102	0.90	106.31
15	BGB107	91.58	BGB107	0.84	99.81	BGB107	0.80	94.99
16	BGB089	77.86	BGB089	0.67	78.76	BGB089	0.67	78.75
17	BGB096	77.52	BGB096	0.66	78.30	BGB096	0.66	77.78
18	BGB088	67.16	BGB088	0.59	70.04	BGB088	0.56	66.24
19	BGB093	61.25	BGB093	0.54	63.75	BGB093	0.51	60.45
20	BGB091	44.93	BGB091	0.38	45.36	BGB091	0.38	45.26
21	BGB086	39.03	BGB086	0.35	41.96	BGB086	0.34	40.38
DSW-Rank	**Genotype**	**HMGV**	**Genotype**	**RPGV**	**RPGV*GM**	**Genotype**	**HMRPGV**	**HMRPGV*GM**
1	BGB467	32.97	BGB467	1.72	33.16	BGB467	1.72	33.16
2	BGB451	24.34	BGB451	1.27	24.47	BGB451	1.27	24.47
3	BGB098	23.72	BGB098	1.24	23.84	BGB098	1.24	23.84
4	BGB101	22.63	BGB101	1.18	22.75	BGB101	1.18	22.75
5	BGB444	22.04	BGB444	1.15	22.13	BGB444	1.15	22.12
6	BGB109	21.67	BGB109	1.13	21.78	BGB109	1.13	21.78
7	BGB083	20.58	BGB083	1.07	20.66	BGB083	1.07	20.65
8	BGB113	20.24	BGB113	1.06	20.35	BGB113	1.06	20.35
9	BGB103	19.70	BGB045	1.03	19.88	BGB045	1.03	19.81
10	BGB045	19.63	BGB103	1.03	19.79	BGB103	1.03	19.79
11	BGB472	18.70	BGB472	0.98	18.80	BGB472	0.98	18.80
12	BGB093	18.60	BGB093	0.97	18.68	BGB093	0.97	18.64
13	BGB089	18.01	BGB089	0.94	18.09	BGB089	0.94	18.08
14	BGB102	17.78	BGB102	0.93	17.88	BGB102	0.93	17.88
15	BGB009	17.67	BGB009	0.92	17.79	BGB009	0.92	17.79
16	BGB107	16.83	BGB107	0.88	16.92	BGB107	0.88	16.91
17	BGB096	15.21	BGB096	0.79	15.27	BGB096	0.79	15.24
18	BGB088	14.77	BGB088	0.77	14.83	BGB088	0.77	14.79
19	BEL	14.37	BEL	0.75	14.49	BEL	0.75	14.47
20	BGB091	13.11	BGB091	0.69	13.29	BGB091	0.69	13.24
21	BGB086	9.55	BGB086	0.50	9.68	BGB086	0.50	9.64
NBT-Rank	**Genotype**	**HMGV**	**Genotype**	**RPGV**	**RPGV*GM**	**Genotype**	**HMRPGV**	**HMRPGV*GM**
1	BGB113	13.01	BGB113	2.75	13.93	BGB113	2.74	13.93
2	BGB088	11.24	BGB088	2.39	12.14	BGB088	2.39	12.12
3	BGB093	9.85	BGB093	2.04	10.35	BGB093	2.02	10.26
4	BGB102	8.62	BGB102	1.81	9.19	BGB102	1.81	9.19
5	BGB103	6.62	BGB103	1.42	7.21	BGB103	1.41	7.17
6	BGB472	6.41	BGB472	1.34	6.78	BGB472	1.33	6.76
7	BGB451	5.01	BGB451	1.05	5.33	BGB109	1.02	5.18
8	BGB091	4.79	BGB109	1.04	5.28	BEL	1.00	5.05
9	BGB109	4.69	BEL	1.02	5.19	BGB451	0.99	5.01
10	BEL	4.56	BGB091	0.99	5.02	BGB091	0.97	4.93
11	BGB101	4.34	BGB107	0.93	4.70	BGB101	0.88	4.45
12	BGB107	3.86	BGB101	0.90	4.56	BGB107	0.86	4.39

13	BGB444	3.71	BGB444	0.82	4.14	BGB444	0.71	3.59
14	BGB089	2.83	BGB089	0.72	3.63	BGB089	0.64	3.26
15	BGB083	2.68	BGB083	0.56	2.83	BGB083	0.53	2.71
16	BGB098	2.46	BGB098	0.52	2.66	BGB098	0.52	2.65
17	BGB086	1.25	BGB086	0.27	1.37	BGB086	0.27	1.36
18	BGB096	1.13	BGB096	0.24	1.24	BGB096	0.22	1.10
19	BGB467	0.30	BGB467	0.09	0.46	BGB467	0.07	0.36
20	BGB009	-0.18	BGB009	0.05	0.27	BGB009	-0.05	-0.27
21	BGB045	-0.18	BGB045	0.05	0.27	BGB045	-0.05	-0.27
NST-Rank	**Genotype**	**HMGV**	**Genotype**	**RPGV**	**RPGV*GM**	**Genotype**	**HMRPGV**	**HMRPGV*GM**
1	BGB113	22.15	BGB113	3.19	22.98	BGB113	3.18	22.88
2	BGB088	20.37	BGB088	2.94	21.19	BGB088	2.85	20.55
3	BGB102	13.09	BGB102	1.90	13.68	BGB102	1.90	13.67
4	BGB101	8.66	BGB109	1.69	12.17	BGB109	1.35	9.71
5	BGB109	8.46	BGB107	1.47	10.56	BGB101	1.26	9.10
6	BGB451	8.07	BGB451	1.27	9.13	BGB107	1.10	7.94
7	BGB103	7.03	BGB101	1.26	9.10	BGB451	1.08	7.75
8	BGB107	6.85	BGB103	1.06	7.61	BGB103	1.04	7.53
9	BGB444	5.42	BGB444	0.80	5.77	BGB444	0.74	5.35
10	BGB098	5.21	BGB098	0.76	5.50	BGB098	0.72	5.18
11	BGB083	4.71	BGB083	0.71	5.10	BGB083	0.64	4.61
12	BGB096	4.19	BGB093	0.66	4.75	BGB096	0.62	4.48
13	BGB093	4.06	BGB096	0.63	4.52	BGB472	0.55	3.98
14	BGB472	3.61	BGB472	0.59	4.24	BGB093	0.54	3.86
15	BGB089	2.82	BGB089	0.42	3.04	BGB089	0.42	3.01
16	BEL	2.78	BEL	0.40	2.89	BEL	0.40	2.87
17	BGB091	2.19	BGB091	0.32	2.33	BGB091	0.32	2.32
18	BGB086	2.14	BGB086	0.31	2.22	BGB086	0.30	2.20
19	BGB467	1.58	BGB467	0.28	2.05	BGB467	0.25	1.78
20	BGB045	0.95	BGB045	0.25	1.82	BGB045	0.16	1.12
21	BGB009	0.33	BGB009	0.09	0.62	BGB009	0.05	0.39
NTT-Rank	**Genotype**	**HMGV**	**Genotype**	**RPGV**	**RPGV*GM**	**Genotype**	**HMRPGV**	**HMRPGV*GM**
1	BGB113	35.97	BGB113	3.08	37.76	BGB113	3.07	37.74
2	BGB088	32.79	BGB088	2.78	34.16	BGB088	2.76	33.88
3	BGB102	21.98	BGB102	1.89	23.20	BGB102	1.89	23.20
4	BGB093	14.37	BGB109	1.42	17.49	BGB103	1.19	14.64
5	BGB103	13.55	BGB107	1.24	15.22	BGB109	1.19	14.61
6	BGB101	13.15	BGB093	1.23	15.09	BGB093	1.18	14.45
7	BGB451	13.08	BGB103	1.21	14.81	BGB101	1.12	13.71
8	BGB109	12.67	BGB451	1.20	14.70	BGB451	1.03	12.61
9	BGB472	9.99	BGB101	1.12	13.75	BGB107	0.95	11.66
10	BGB107	9.95	BGB472	0.88	10.85	BGB472	0.88	10.76
11	BGB444	8.91	BGB444	0.81	9.91	BGB444	0.70	8.63
12	BGB098	7.75	BGB098	0.66	8.11	BGB098	0.64	7.82
13	BEL	7.23	BGB083	0.64	7.88	BEL	0.63	7.79
14	BGB083	7.20	BEL	0.64	7.86	BGB091	0.58	7.06
15	BGB091	6.84	BGB091	0.58	7.13	BGB083	0.57	7.02
16	BGB089	5.42	BGB089	0.53	6.45	BGB089	0.49	6.05
17	BGB096	5.34	BGB096	0.46	5.60	BGB096	0.46	5.59
18	BGB086	3.21	BGB086	0.27	3.34	BGB086	0.27	3.31
19	BGB467	1.67	BGB467	0.18	2.21	BGB467	0.16	1.91
20	BGB045	0.49	BGB045	0.14	1.76	BGB045	0.05	0.61
21	BGB009	-0.06	BGB009	0.04	0.53	BGB009	-0.01	-0.08
WBT-Rank	**Genotype**	**HMGV**	**Genotype**	**RPGV**	**RPGV*GM**	**Genotype**	**HMRPGV**	**HMRPGV*GM**
1	BGB091	103.04	BGB091	2.26	114.04	BGB091	2.20	111.13
2	BGB113	84.73	BGB113	1.88	95.10	BGB113	1.87	94.73
3	BGB472	81.80	BGB093	1.87	94.43	BGB093	1.85	93.42
4	BGB093	78.82	BGB088	1.83	92.76	BGB088	1.80	91.09
5	BGB088	76.06	BEL	1.82	92.16	BGB472	1.77	89.52
6	BEL	72.83	BGB472	1.80	90.84	BEL	1.76	88.85
7	BGB102	69.10	BGB102	1.52	76.97	BGB102	1.51	76.20
8	BGB103	51.08	BGB103	1.21	61.11	BGB103	1.20	60.49
9	BGB451	49.61	BGB451	1.09	55.17	BGB451	1.02	51.64
10	BGB109	39.68	BGB089	1.09	55.06	BGB109	0.88	44.29
11	BGB101	34.96	BGB109	0.88	44.49	BGB089	0.83	42.17
12	BGB089	31.49	BGB101	0.76	38.68	BGB101	0.74	37.44
13	BGB444	31.24	BGB444	0.76	38.64	BGB444	0.59	29.59
14	BGB098	24.07	BGB107	0.68	34.44	BGB107	0.57	29.02
15	BGB107	22.26	BGB098	0.53	26.85	BGB098	0.53	26.62
16	BGB083	19.26	BGB083	0.43	21.70	BGB083	0.39	19.60
17	BGB086	10.44	BGB086	0.26	12.98	BGB086	0.25	12.63

18	BGB096	7.00	BGB096	0.15	7.77	BGB096	0.15	7.34
19	BGB467	2.17	BGB467	0.07	3.79	BGB467	0.06	2.90
20	BGB009	-0.92	BGB009	0.05	2.42	BGB009	-0.03	-1.53
21	BGB045	-0.92	BGB045	0.05	2.42	BGB045	-0.03	-1.53
WST-Rank	**Genotype**	**HMGV**	**Genotype**	**RPGV**	**RPGV*GM**	**Genotype**	**HMRPGV**	**HMRPGV*GM**
1	BGB113	28.80	BGB113	2.53	30.73	BGB113	2.45	29.76
2	BGB088	25.32	BGB088	2.22	26.99	BGB088	2.17	26.36
3	BGB102	22.91	BGB102	2.02	24.58	BGB102	2.01	24.47
4	BGB109	15.93	BGB109	1.58	19.23	BGB109	1.51	18.38
5	BGB107	14.67	BGB107	1.50	18.30	BGB107	1.41	17.12
6	BGB098	13.76	BGB098	1.41	17.09	BGB098	1.32	16.04
7	BGB103	13.74	BGB103	1.23	14.99	BGB103	1.23	14.99
8	BGB451	12.97	BGB451	1.17	14.29	BGB451	1.06	12.84
9	BGB101	10.05	BGB101	0.96	11.63	BGB101	0.94	11.39
10	BGB444	9.98	BGB444	0.88	10.72	BGB472	0.84	10.19
11	BGB472	9.40	BGB472	0.84	10.19	BGB444	0.84	10.16
12	BGB083	7.69	BGB083	0.67	8.20	BGB083	0.66	7.98
13	BGB096	6.82	BGB096	0.61	7.45	BGB096	0.61	7.44
14	BEL	6.81	BEL	0.60	7.31	BEL	0.60	7.29
15	BGB091	6.03	BGB091	0.55	6.66	BGB091	0.55	6.65
16	BGB089	5.81	BGB089	0.54	6.51	BGB089	0.53	6.47
17	BGB093	5.17	BGB093	0.46	5.56	BGB093	0.46	5.55
18	BGB086	4.35	BGB086	0.40	4.87	BGB086	0.40	4.84
19	BGB467	2.88	BGB467	0.34	4.13	BGB467	0.29	3.48
20	BGB045	2.23	BGB045	0.28	3.41	BGB045	0.23	2.74
21	BGB009	1.64	BGB009	0.22	2.63	BGB009	0.17	2.03
WTT-Rank	**Genotype**	**HMGV**	**Genotype**	**RPGV**	**RPGV*GM**	**Genotype**	**HMRPGV**	**HMRPGV*GM**
1	BGB113	119.23	BGB113	2.10	131.51	BGB113	2.07	130.07
2	BGB091	108.84	BGB088	1.99	124.55	BGB088	1.98	124.19
3	BGB088	107.80	BGB091	1.91	119.56	BGB091	1.87	117.01
4	BGB102	94.59	BGB102	1.67	104.64	BGB102	1.66	103.86
5	BGB472	91.37	BGB472	1.61	100.73	BGB472	1.59	99.55
6	BGB093	84.26	BGB093	1.58	99.14	BGB093	1.57	98.30
7	BEL	80.51	BEL	1.58	99.14	BEL	1.54	96.32
8	BGB103	65.35	BGB103	1.22	76.81	BGB103	1.21	76.19
9	BGB451	63.38	BGB451	1.13	71.11	BGB451	1.02	63.95
10	BGB109	54.33	BGB109	1.02	63.83	BGB109	1.01	63.33
11	BGB101	44.67	BGB089	0.97	60.72	BGB101	0.78	49.18
12	BGB444	40.12	BGB107	0.84	52.68	BGB089	0.77	48.59
13	BGB089	37.28	BGB101	0.79	49.49	BGB098	0.68	42.61
14	BGB098	35.96	BGB444	0.79	49.45	BGB107	0.68	42.42
15	BGB107	32.59	BGB098	0.69	43.45	BGB444	0.60	37.92
16	BGB083	25.73	BGB083	0.47	29.20	BGB083	0.41	25.64
17	BGB086	13.62	BGB086	0.26	16.04	BGB086	0.25	15.90
18	BGB096	12.69	BGB096	0.22	13.94	BGB096	0.21	13.36
19	BGB467	2.46	BGB467	0.09	5.51	BGB467	0.05	3.38
20	BGB045	-1.45	BGB045	0.05	3.32	BGB045	-0.04	-2.37
21	BGB009	-1.90	BGB009	0.04	2.46	BGB009	-0.05	-3.27
DMC-Rank	**Genotype**	**HMGV**	**Genotype**	**RPGV**	**RPGV*GM**	**Genotype**	**HMRPGV**	**HMRPGV*GM**
1	BGB451	28.65	BGB451	1.39	28.80	BGB451	1.39	28.78
2	BGB444	27.91	BGB444	1.35	28.05	BGB444	1.35	28.04
3	BGB102	27.80	BGB102	1.35	27.95	BGB102	1.35	27.93
4	BGB103	26.83	BGB103	1.30	26.98	BGB103	1.30	26.97
5	BGB083	26.24	BGB083	1.27	26.38	BGB083	1.27	26.37
6	BGB113	26.17	BGB113	1.27	26.31	BGB113	1.27	26.31
7	BGB109	26.11	BGB109	1.27	26.25	BGB109	1.27	26.24
8	BGB101	25.86	BGB101	1.25	26.01	BGB101	1.25	26.00
9	BGB107	25.43	BGB107	1.23	25.57	BGB107	1.23	25.57
10	BGB472	24.56	BGB472	1.19	24.70	BGB472	1.19	24.69
11	BGB098	24.24	BGB098	1.18	24.38	BGB098	1.18	24.38
12	BEL	20.01	BEL	0.97	20.15	BEL	0.97	20.15
13	BGB093	19.17	BGB093	0.93	19.30	BGB093	0.93	19.30
14	BGB089	18.46	BGB089	0.90	18.60	BGB089	0.90	18.60
15	BGB096	16.32	BGB096	0.79	16.46	BGB096	0.79	16.46
16	BGB086	15.36	BGB086	0.75	15.50	BGB086	0.75	15.49
17	BGB091	15.27	BGB091	0.74	15.42	BGB091	0.74	15.41
18	BGB088	14.36	BGB088	0.70	14.52	BGB088	0.70	14.50
19	BGB467	12.66	BGB467	0.62	12.83	BGB467	0.62	12.80
20	BGB045	9.01	BGB045	0.45	9.28	BGB045	0.44	9.15
21	BGB009	1.07	BGB009	0.10	2.14	BGB009	0.05	1.14

CHAPTER III

3. Evaluating potato wild relatives (*Solanum* sect. *Petota*, Solanaceae) germplasm diversity for photosynthetic traits response under heat stress[3]

3.1. Abstract

Heat stress is one of the major factors reducing the 3rd most important food crop by limiting the plant photosynthesis activity (particularly PSII), limiting the production, and partitioning of assimilates to the sink (tuber). Wild potato germplasm serves as a natural reservoir of valuable traits for potato breeding, such as abiotic stress resistance. However, it is necessary to identify phenotypes on a physiological basis that could be manipulated to increase crop photosynthesis. Thus, the traits of gas exchange, chlorophyll index and chlorophyll fluorescence are important to study because these factors mainly influence photosynthetic activity. Under heat stress, gas exchange and chlorophyll fluorescence of studied wild potato genotypes responded diversly. Further statistical analysis indicates that it is possible to characterize the wild genotypes performing better in high temperatures. Most important traits needed to be focused are effective photochemical yield, non-photochemical quenching, Fv/Fm ratio and there is positive relationship with net photosynthetic rates, transpiration rates, and stomatal conductance. As a result of this study, we can conclude that wild genotypes, despite their diverse distribution in different climates, are beneficial. It is possible to group them based on the traits evaluated in this study. Furthermore, *S. commersonii* accessions collected from the vicinity of Brazil are more likely to be tolerant to heat stress and followed by the *S. chacoense*. So, introducing the characterized genotypes with improved photosynthesis traits in adverse conditions is important to breeding program but will require continued efforts to improve yield because of difficulties present of introducing wild genotypes.

Keywords: Wild potatoes; heat stress; gas exchange; chlorophyll fluorescence; non-invasive phenotyping; *Solanum chacoense; Solanum commersonii*

[3] The structure of this articles is prepared according to the Plant Physiology Journal (ISSN:1532-2548).

3.2. Introduction

Domestication bottleneck (SHEPHERD *et al.*, 2016) is the main phenomenon responsible for negative impacts of germplasm genetic diversity (HARDIGAN *et al.*, 2017; SMITH *et al.*, 2019) but now recently come under improved screening to minimize these cons. Regardless of the process involved, the germplasm of most crop wild relatives (CWR) be found to show significant variation for photosynthetic traits. So, it is very important to search the allelic diversity within CWR for a climate proof breeding program because apparently genetic diversity within a crop's germplasm is significantly less than that of its wild progenitors. Many researchers dive into to explore the great potential of novel allelic variation in photosynthetic traits, (SHARWOOD *et al.*, 2022) try to find the variation leverage to speed up breeding program progress in wild relatives of fiber crops. African rice is an example of harboring complex traits which enable plant growth and sustainability under stress conditions as reported by (COWLING *et al.*, 2022).

Photosynthesis is a vital and unique natural process that involves several complex processes related to the conversion of solar radiation and its transformation into the energy of chemical bonds, which occurs by photochemical processes powering CO_2 fixation through biochemical reactions and the formation of assimilates as well. As a result, the success of crop species and genotypes is determined by the efficiency of the photosynthetic apparatus (BRESTIC *et al.*, 2018; HUSSAIN *et al.*, 2021; LONG; MARSHALL-COLON; ZHU, 2015). Plant regulatory mechanisms have evolved to adapt to various climatic changes, such as fluctuating irradiation, limited and excess precipitation, or low and high temperatures, during evolutionary processes. Despite the inhibition of their photosynthetic processes, these adaptations allow the plants to thrive even in rapidly changing climatic conditions. As a result, the environment is frequently regarded as a complex factor that influences or limits crop growth and production processes (SHARKEY, 2005; YEH *et al.*, 2012; ZIVCAK; OLSOVSKA; BRESTIC, 2017).

Temperature is an important environmental factor that is directly related to the geographical distribution, survival, and production of plants. In other words, as the temperature approaches limit of tolerance, it inhibits cell homeostasis, impairs the growth and development of living organisms, and, in extreme cases, leads to death. Furthermore, environmental stress such as high temperature can directly or indirectly damage photosynthetic apparatus such as photosystem II (PSII) (NISHIYAMA; ALLAKHVERDIEV; MURATA, 2006; TAKAHASHI; MURATA, 2008), impacting on electron transport through the reaction center of photosystem-

II (PSII).

When considering CO_2 assimilation and electron transport, rates are highly responsive to environments changes which include a series of biophysical, chemical, and physical processes that operate on different timescales. This complex and non-linear set of processes could lead the photosynthetic leaf to experience surplus or a deficit, making a requirement of regulatory processes that contribute to limit over-reduction or over-oxidation of key steps during the dynamic environment changes.

Chlorophyll fluorescence analysis is used in various ways to investigate the response of Photosystem II, and it provides various information on the physiological response of plants to environmental stress and the photosynthetic machinery (STRASSERF; SRIVASTAVA; GOVINDJEE, 1995). The OKJIP curve (KALACHANIS; MANETAS, 2010; RIPOLL *et al.*, 2016; STIRBET; GOVINDJEE, 2011) is a chlorophyll fluorescence curve showing the density of fluorescence over time of fluorescence emitted from leaves irradiated with light. Various information such as change and reduction of electron acceptor can be obtained quantitatively (STRASSER; SRIVASTAVA; TSIMILLI-MICHAEL, 2000).

HAVAUX, (1992) stated that exposure of potato (*Solanum tuberosum* L., Solanaceae) leaves to heat stress (HS) caused an increase in activity of PSII in water-stressed plants, as indicated by a slight increase in variable to maximum chlorophyll fluorescence (Fv/Fm). Additionally, has been recently postulated that non-photochemical quenching (NPQ) plays an important role in determining plant productivity (DEMMIG-ADAMS *et al.*, 2014). According to recent studies, NPQ may contribute to enhance productivity by decreasing the photoinhibition (HUBBART *et al.*, 2018), but may also reduce the plant yield by this overly protective persistence, lowering quantum yield when plant is submitted to low light unnecessarily (KROMDIJK *et al.*, 2016).

The increase in average global temperature is modeled to reach about 0.3-4.8ºC by the end of the XXI century (IPCC, 2021; O'NEILL *et al.*, 2017) which directly affects the growth and development of plants by changing photosynthesis and growth period of plants. This scenario will lead agriculture to a challenge, especially in arid regions.

Potato (*Solanum tuberosum* L.) is cultivated worldwide and considered the third most important food crop, after rice and wheat (BIRCH *et al.*, 2012). Potato is a cool season crop. The optimum temperature range for potato tuberization and tuber growth is 15ºC - 20ºC while aerial parts are well developed between 20ºC - 25°C (RYKACZEWSKA, 2013, 2015). The effect of increasing temperatures above normal optimum is sensed as heat stress in potato and causes physiological damage and ultimately causes tuber yield reduction.

The potato has more than 100 wild relatives in its gene pool (SPOONER *et al.*, 2014). These wild potatoes are widely distributed (HIJMANS; SPOONER, 2001) and considered as a valuable source of genetic variability for potato breeding (BASHIR; NICOLAO; HEIDEN, 2021; HAWKES, 1958; JANSKY *et al.*, 2013). This genetic diversity present in potato wild relatives represents an important role for the continuous process of plant breeding to face climate changes and to ensure global food security. To identify heat tolerance is necessary to evaluate genotypes in a heat stress environment and select those that have superior values of traits of interest as to compare to cultivars with good performance (WAHID *et al.*, 2007). Recently, studies on potato crop cultivation area changes and the development of a yield change prediction model have been actively conducted to understand the response of plants to climate change (JENNINGS *et al.*, 2020).

Increasing the high-temperature tolerance of crops is one of the critical challenges facing plant research and breeding practices. Climate change, especially the increase of global 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 and photochemical and non-photochemical quenching of chlorophyll fluorescence may help to identify and phenotype, aiming define new plant ideotype to face future climate scenarios. Thus, the aim of this study was to evaluate photosynthetic traits of wild germplasm from the Potato Genebank of Embrapa (Brazilian Agricultural Research Corporation) under control and heat stress treatments.

3.3. Material and methods

The experiment was carried out in controlled environment, at the Phenotyping platform from Embrapa Clima Temperado, in Pelotas, Rio Grande do Sul, Brazil (32º45´ S and 52º30´ W), between January to April 2019. Tubers of uniform sizes from 24 accessions (Table 1) selected from Embrapa's 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; HEILBRONN, 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.*, 2021). The photoperiod was 12 hours (7:00 to 19:00h) with a light intensity of 400 μmol $m^{-2}s^{-1}$, approximately (**Figure 1**). The

evaluated plants remained in these conditions until the time of harvest at 84 days after planting, totaling 62 days of exposure to temperature treatment. The data were recorded according to factorial experimental design (**Figure 11**).

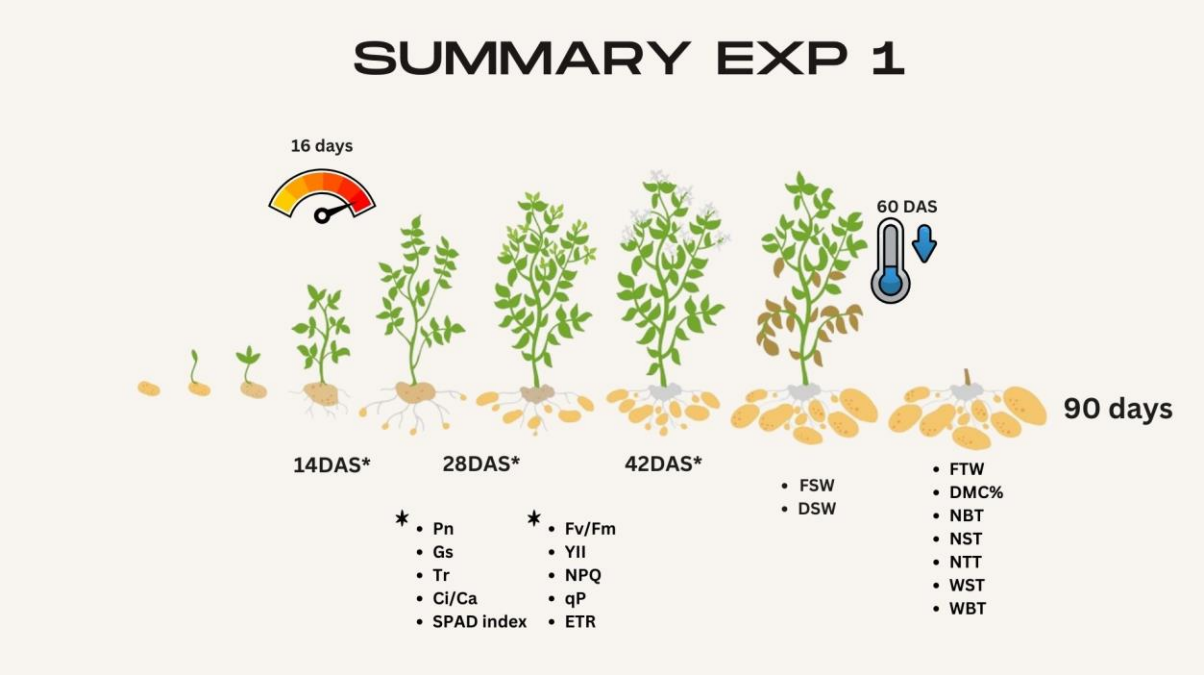

Figure 11: Graphical summary of experiment 1 conducted during January-April 2019.

3.3.1. 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. The initial fluorescence (Fo) in the open centers of the photosystem-II (PSII) was determined by measuring light (less than 30 μmol $m^{-2}s^{-1}$), while the maximum fluorescence (Fm) in closed centers or in a state Reduced PSII was evaluated after the application of a pulse of 0.8 seconds of saturation light (7000 μmol $m^{-2}s^{-1}$). The induction curves were started by a pulse of saturation light applied every 20 seconds until reaching steady state condition. During measurements, the actinic light (red light) was activated to quantify the steady state of chlorophyll fluorescence. In plants adapted to light, Fm' was analyzed through the application of saturated pulse, while Fo' was evaluated by turning off the actinic light for 2 seconds after the saturation pulse and lighting the red-distant light. For statistical analysis, we used the parameters Fv/Fm, Y(II), NPQ, qN and ETR derived from the equations described in **Table III**, to which mean values correspond to 281, 336, 396 and 461 μmol $m^{-2}s^{-1}$ PAR.

Table 4: Chlorophyll fluorescence parameters analyzed. Abbreviations and meanings of the variables of photosystem II photochemistry produced by the PAM-2500 portable fluorometer (Walz, Germany).

Parameter	Equations	Physiological relevance
Fv/Fm	$(\boldsymbol{F_m - F_0}) \,/\, \boldsymbol{F_m}$	Maximum photochemical quantum yield of PSII (KITAJIMA; BUTLER, 1975)
Y(II)	$(\boldsymbol{Fm' - F})/\boldsymbol{Fm'}$	Effective photochemical quantum yield of PSII (GENTY; BRIANTAIS; BAKER, 1989)
NPQ	$(\boldsymbol{Fm - Fm'})/\boldsymbol{Fm'}$	Nonphotochemical quenching used to monitors the apparent rate constant for heat loss from PSII. (BILGER; BJÖRKMAN, 1990).
qP	$(\boldsymbol{Fm' - F})/(\boldsymbol{Fm' - Fo'})$	Coefficient of photochemical fluorescence quenching, ranging from 0 (upon application of a Saturation Pulse) to 1 (in the dark-acclimated state). (SCHREIBER; SCHLIWA; BILGER, 1986) as formulated by(VAN KOOTEN; SNEL, 1990)
ETR	0.5×Yield×PAR×0.84µequivalent $m^{-2}s^{-1}$	Electron transport rate

3.3.2. Leaf gas exchange

Measurements were performed by using the LI-6400XT photosynthesis system (LiCor, Lincoln, NE, USA) mounted with a red/blue LED light source (6400-02B; Li-Cor) 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 and same temperature settings were fixed in the photosynthesis system analyzer, respectively. The CO_2 concentration used in the chamber was 400 µmol mol^{-1} and a photon flux density of 400 µmol of photons $m^{-2}s^{-1}$, and relative humidity 50-65%. Net photosynthesis rate (Pn) µmol C_2O $m^{-2}s^{-1}$, stomatal conductance (Gs) mol H_2O $m^{-2}s^{-1}$, transpiration (E) µmol H_2O $m^{-2}s^{-1}$, and intracellular and ambient CO_2 assimilation ratios (C*i*/C*a*) were calculated according to (VON CAEMMERER; FARQUHAR, 1981) with the software of the Li6400.

Water-use efficiency (WUE) is a measure of the carbon gained by plants through photosynthesis relative to the water lost through transpiration, defined as $\frac{Pn}{E}$ where Pn is net photosynthesis and E is transpiration. This is commonly referred to as instantaneous WUE (Farquhar & Richards, 1984). However, instantaneous WUE can depend on environmental conditions, as differences in the vapor pressure of water in the air can vary significantly and lead to significant differences in E. To improve the ability to compare across studies without these confounding effects, intrinsic WUE (iWUE; Osmond et al., 1980) was proposed, defined as $\frac{Pn}{Gs}$. Both WUE and iWUE are measured in µmol CO_2 mol^{-1} H_2O (Medrano et al 2015).

3.3.3. 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).

3.3.4. Statistical analysis:

Statistical analyses were performed for screening of heat tolerant accessions following evaluations performed on 14, 28 and 42 days after stress (DAS) for chlorophyll fluorescence (CF), gas exchange parameters and chlorophyll index. The recorded data was pooled and considered as replicated to perform the factorial analysis of variance by using "easyanova" (ARNHOLD, 2013) package. Principle component analysis and clustering was performed by "FactoMineR" (LÊ; JOSSE; HUSSON, 2008) and "Factoextra" package (KASSAMBARA; MUNDT, 2021). Correlation was done by "corrplot" (WEI; SIMKO, 2017) and "ggcorrplot" (KASSAMBARA; KASSAMBARA, 2019). The figures were developed by using "patchwork" package (PEDERSEN, 2020). All analysis were carried out in R Studio (KRONTHALER; ZÖLLNER, 2021) and "Rprogram" (YEH *et al.*, 2012).

Cluster score was calculated as a weighted linear combination of physiological traits to designate the cluster based on their tolerance level (*i.e.*, summation of weightage multiplied with their respective physiological trait).

Cluster score = $\sum_{i=1}^{n} X_i W_i$

Where X*i* is the mean value of the *i*th physiological trait in the given cluster and W*i* is the weightage associated with the *i*th physiological trait in the given cluster. Weightage was obtained from PCA analysis of communities. Clusters were classified as tolerant, intermediate, or sensitive based on their cluster score.

3.4. Results

Analysis of variance (ANOVA) results for all examined traits is presented in **Table 2**, 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 (Figure 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 (Figure 1). The mean values of all traits measured on 14, 28 and 42 days after stress in both control and stress conditions.

Table 5: Analysis of variance (ANOVA) of all studied traits under normal (CT) and Heat Stress (HS) conditions.

SOV	DF	SPAD	Pn	Gs	E	WUE	iWUE	C*i*/C*a*	Y(II)	NPQ	qP	ETR	Fv/Fm
Genotype (G)	23	0.000^{***}	0.000^{***}	0.001^{***}	0.005^{**}	0.436^{ns}	0.059^{ns}	0.391^{ns}	0.000^{***}	0.014^{**}	0.000^{***}	0.000^{***}	0.000^{***}
Treatment (T)	1	0.000^{***}	0.037^{*}	0.000^{***}	0.740^{ns}	0.000^{***}	0.000^{***}	0.000^{***}	0.073^{ns}	0.009^{**}	0.003^{**}	0.070^{ns}	0.000^{***}
DAS (Rep)	2	0.000^{***}	0.001^{***}	0.001^{***}	0.074^{ns}	0.314^{ns}	0.237^{ns}	0.119^{ns}	0.000^{***}	0.213^{ns}	0.000^{***}	0.000^{***}	0.000^{***}
G × T	23	0.06^{ns}	0.804^{ns}	0.873^{ns}	0.455^{ns}	0.054^{ns}	0.863^{ns}	0.462^{ns}	0.757^{ns}	0.801^{ns}	0.651^{ns}	0.756^{ns}	0.536^{ns}

ns P > 0.05, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001

3.4.1. 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 (**Supplementary Table T1)**. 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 T1**).

The net photosynthesis rate (Pn) of potato germplasm under control condition ranged from 3.99-15.00 with a mean rate of 9.89 µmol CO_2 $m^{-2}s^{-1}$. In HS conditions, it ranged from 5.41-17.53 with an average of 11.15. Under control temperature conditions, *S. commersonii* BGB009 showed the highest while *S. chacoense* BGB102 showed the lowest values for net rate of photosynthesis. In stress conditions (HS), *S. tuberosum* 2x BGB091 attains the maximum values and minimum photosynthesis rate and genotype BGB083 exhibits the minimum values (**Supplementary Table T1**).

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 mol H_2O $m^{-2}s^{-1}$ under stress conditions (HS) as listed in **Supplementary Table T1**. 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 T1**).

The transpiration rate (E) of the given data had mean values of 4.42 and 4.34 mmol H_2O m^{-2} s^{-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 T1**).

Solanum commersonii genotypes BGB045 and BGB003, showed the high intracellular and ambient CO_2 assimilation ratio (C*i*/C*a*) under both applied conditions, while *S. chacoense* BGB109 was negative under stress and *S. tuberosum* 2x BGB093 had low C*i*/C*a* in control condition, respectively (**Supplementary Table T1**). Observed values for CO_2 assimilation ratio (C*i*/C*a*) under CT and HS conditions have significant differences. Mean values under control was 0.82 and for stress was 0.43 **(Supplementary Table T1)**.

Water use efficiency (WUE) and intrinsic water use efficiency (iWUE) (**Supplementary Table T1**) 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.

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 (**Supplementary Table T1)**. *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 T1**).

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 T1**). 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 T1**).

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 T1**). 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 T1)**.

The violin plot graph in **Figure 12 A-L** 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 CO_2 assimilation ratio (C*i*/C*a*), effective photochemical quantum yield of PS-II (YII), coefficient of photochemical quenching (qP) and high electron transport rates (ETR). *Solanum chacoense* showed high water use effciency (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 C*i*/C*a*. *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 **Figure 12.**

The mean performance of potato germplasm (**Table 1.**) under both treatments (CT and HS) for all studied traits on 14 days after stress (DAS), 28DAS and 42DAS are showed in **Figure 16**. From box plot we can clearly observe that for all studied traits there was a declining trend in the observation with passage of crop cycle and analysis of variance (ANOVA). **Table 2** for days after stress showed significant differences for SPAD, Pn, Gs, E, C*i*/C*a* and Fv/Fm

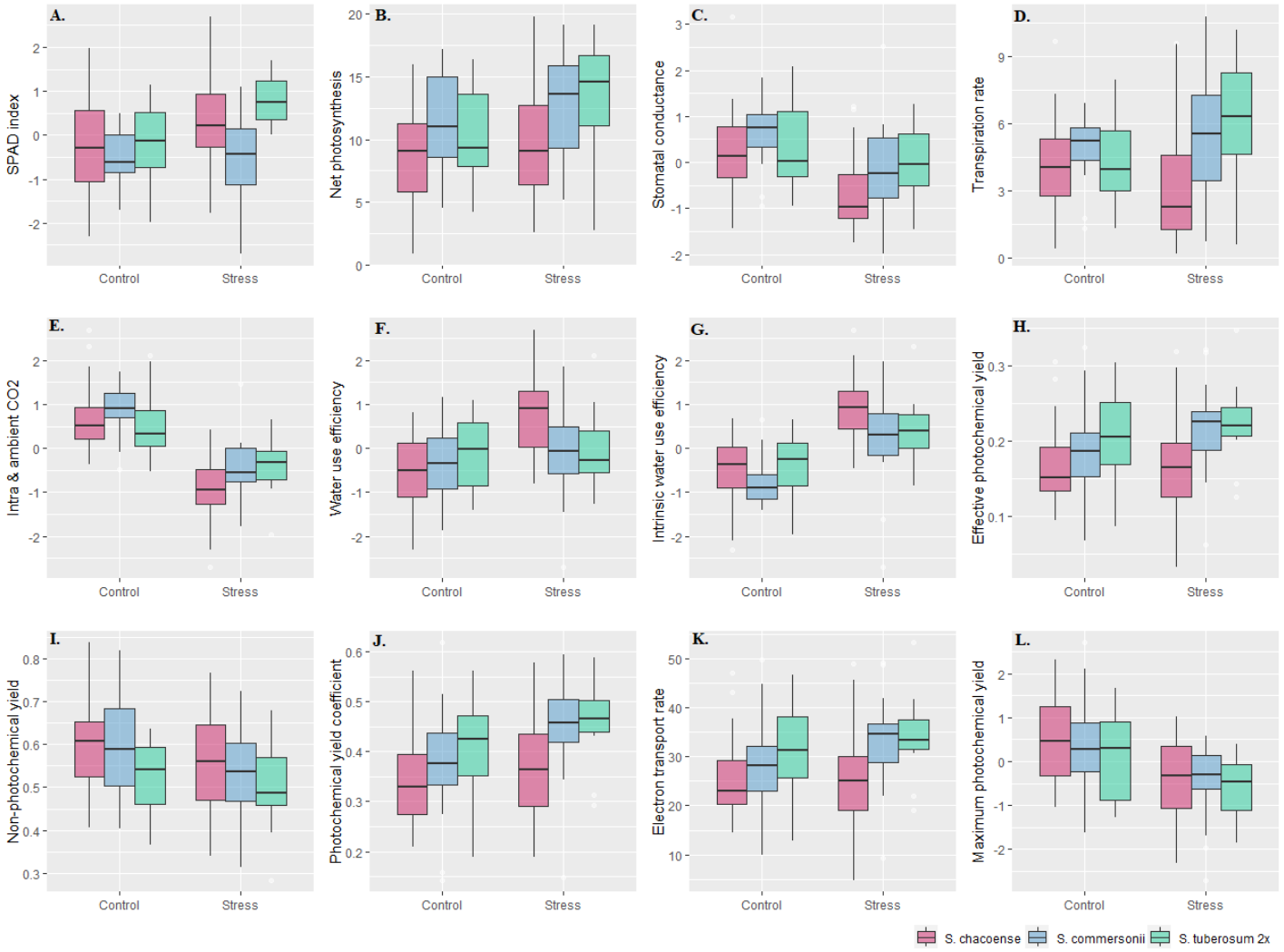

Figure 12: Performance wild and cultivated potatoes (*S. chacoense*, *S. commersonii*, *S. tuberosum* 2x) under control and heat stress condition for A-SPAD index (spad), B-net photosynthesis rate (Pn), C-stomatal conductance (Gs), D-transpiration rate (E), E-internal & ambient CO2 assimilation (Ci/Ca), F-water use efficiency (WUE), G-intrinsic water use efficiency (iWUE), H-effective photochemical yield of photosystem-II (YII), I-non-photochemical yield (NPQ), J-coefficiecnt of photochemical yield (qP), K-ekectron transport rate (ETR) and L-maximum photochemical yield of photosystem II (Fv/Fm)

3.4.2. Correlation among photosynthetic traits

Correlation of 24 genotypes for all studied chlorophyll fluorescence and photosynthesis traits were shown in **Figure 13, Supplementary Table T2** under control temperature (CT) range (**A**) and heat stress (HS) condition (**B**). Net photosynthesis rate (Pn) had positive and highly significant association with the stomatal conductance (Gs), transpiration rate (E), intracellular and ambient CO_2 assimilation ratio (C*i*/C*a*), 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 CO_2 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 C*i*/C*a*.

In 14-24°C treatment (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 **Figure 13 A & B**.

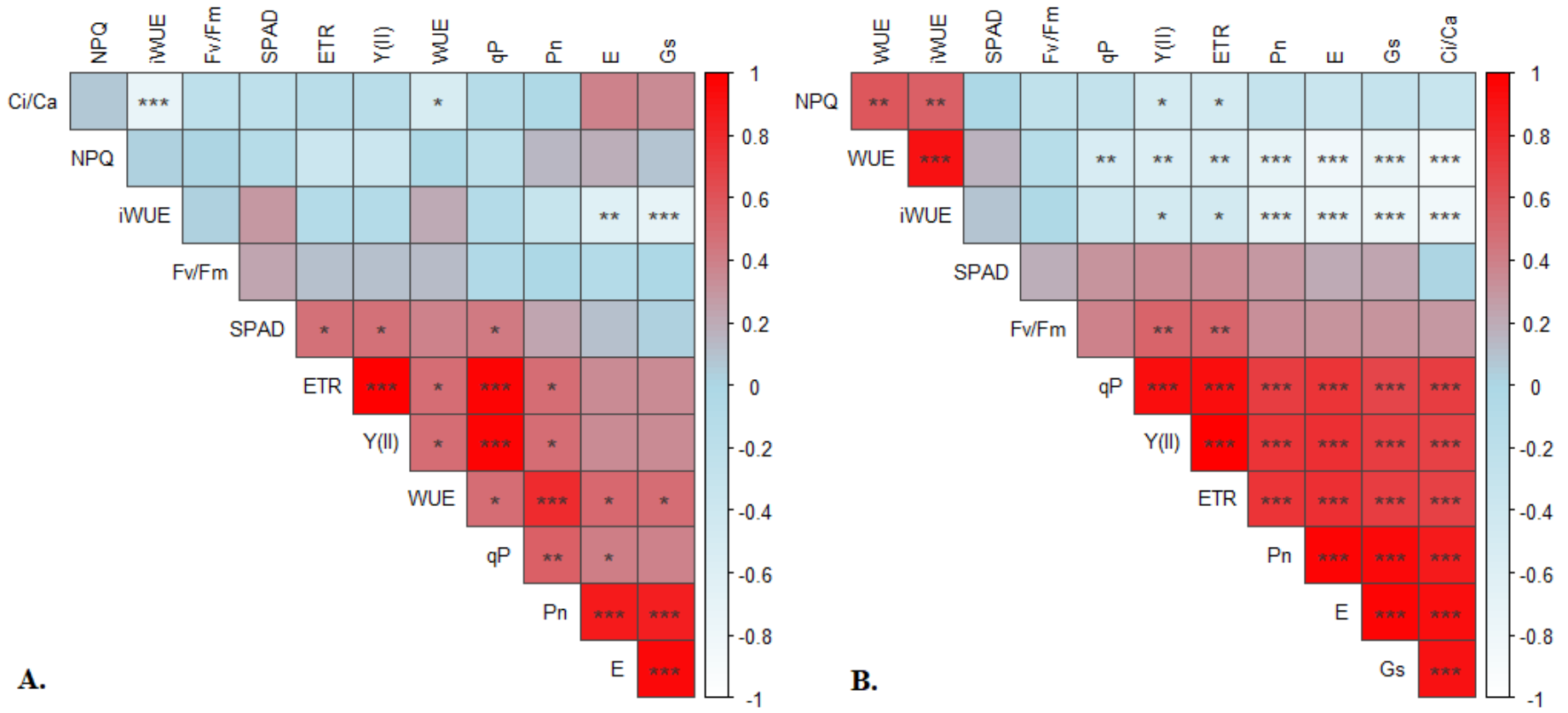

Figure 13: Correlation between different variables of chlorophyll fluorescence and photosynthesis traits in 24 potato (*Solanum* sect. *Petota*, Solanaceae) genotypes leaves. A. Control treatment (CT) 14-27°C, and B. Heat stress (HS) 24-34°C. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

3.4.3. 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 (**Table 7)**. 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, C*i*/C*a*, 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) has the high positive correlation.

Table 6: Eigenvector values of 10 variables, evaluated in potato (*Solanum* sect. *Petota*, Solanaceae) germplasm from Embrapa Potato Genebank under control temperature and heat stress. PC1= first principal component; PC2=second principal component; PC3=third principal component; PC4= third principal component; PC5= fifth principal component; PC6= sixth principal component.

Principal component	PC1		PC2		PC3		PC4		PC5		PC6	
	CT	HS	CT	HS	CT	HS	CT	HS	CT	HS	CT	HS
SPAD	0.44	0.24	0.54	0.74	0.18	0.15	-0.22	-0.23	-0.14	-0.13	0.57	0.43
Pn	0.73	0.92	-0.34	0.09	0.53	0.21	-0.07	0.19	-0.02	-0.01	0.03	-0.02
Gs	0.63	0.93	-0.67	0.00	0.27	0.16	-0.05	0.21	-0.07	-0.07	0.15	-0.05
E	0.61	0.95	-0.64	0.02	0.32	0.08	-0.12	0.18	-0.02	0.02	0.24	-0.07
Ci/Ca	-0.03	0.88	-0.68	-0.18	-0.47	-0.02	-0.08	0.29	0.13	0.05	0.46	-0.13
WUE	0.63	-0.81	0.18	0.35	0.67	0.24	-0.03	-0.09	-0.06	0.05	-0.14	0.18
iWUE	-0.27	-0.81	0.81	0.4	0.31	0.08	-0.03	0.00	0.02	0.24	-0.20	0.12
Y(II)	0.84	0.83	0.22	0.29	-0.15	-0.25	-0.34	0.07	-0.12	0.33	-0.10	0.07
NPQ	-0.18	-0.48	-0.25	0.09	0.43	0.69	0.37	0.17	0.36	0.26	0.26	-0.05
qP	0.81	0.77	0.15	0.29	-0.06	-0.14	-0.41	0.06	0.03	0.52	-0.08	-0.01
ETR	0.84	0.83	0.22	0.29	-0.15	-0.25	-0.34	0.07	-0.13	0.33	-0.10	0.08
Fv/Fm	0.20	0.31	0.25	0.42	-0.07	-0.35	0.68	0.61	-0.59	0.03	0.16	0.10
Eigenvalue	11.18		3.96		2.40		1.74		1.19		1.07	
% Variance	46.60		16.48		9.98		7.24		4.98		4.46	
Cumulative var. %	46.60		63.09		73.07		80.31		85.29		89.75	

The first PC was negatively correlated by C*i*/C*a*, NPQ, iWUE in CT and NPQ, WUE, iWUE under HS. Under CT conditions, PC2 showed negative values for NPQ, Pn, E, Gs and C*i*/C*a* 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 **Figure 14**. 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.

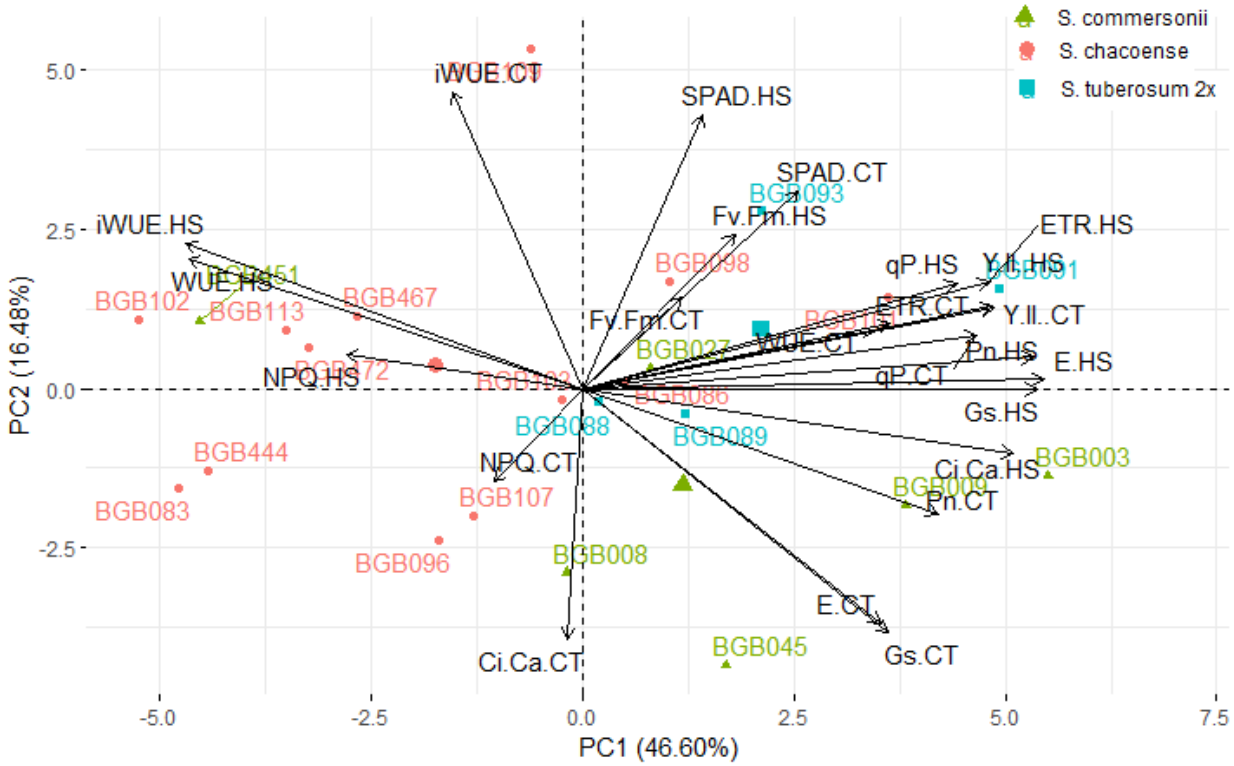

Figure 14: Dispersion of 24 potato (*Solanum* sect. *Petota*, Solanaceae) genotypes from Embrapa Potato Genebank by principal component analysis evaluated under control temperature (CT) and heat stress (HS) conditions, for the SPAD chlorophyll Index (SPAD), net photosynthesis rate (Pn), stomatal conductance (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).

3.4.4. 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 Figure 5. 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 (**Figure 15**) characterized by Pn, Gs, E, WUE, iWUE, C*i*/C*a*, Y(II), qP and ETR (**Supplementary Table 4**). 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, C*i*/C*a*, Y(II) and ETR are most significantly associated with this cluster (**Supplementary Table 4**).

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 8**.

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.

Table 7: Descriptive statistics of physiological traits of potato (*Solanum* sect. *Petota*, Solanaceae) genotypes from Embrapa Potato Genebank for different clusters under control and heat stress condition.

Traits	RI %		Cluster I Score		Cluster II Score		Cluster III Score	
	CT	HS	CT	HS	CT	HS	CT	HS
SPAD	0.91	0.89	-0.65	0.07	-0.16	0.20	0.12	0.21
Pn	0.93	0.94	7.05	6.80	8.72	10.23	13.31	15.77
Gs	0.93	0.94	-0.01	-1.02	0.35	-0.35	0.75	0.52
E	0.96	0.94	3.54	1.63	4.28	4.21	5.07	7.01
C*i*/C*a*	0.93	0.91	0.60	-1.18	0.72	-0.56	0.55	-0.06
WUE	0.90	0.88	-0.59	1.06	-0.4	0.12	0.20	-0.22
iWUE	0.86	0.9	-0.42	1.10	-0.53	0.43	-0.50	0.03
Y(II)	0.92	0.96	0.13	0.13	0.17	0.19	0.19	0.23
NPQ	0.62	0.81	0.37	0.46	0.36	0.42	0.37	0.41
qP	0.85	0.97	0.26	0.31	0.32	0.40	0.36	0.47
ETR	0.92	0.96	20.29	20.97	25.70	28.84	29.6	35.34
Fv/Fm	0.93	0.77	0.34	-0.39	0.35	-0.38	0.47	-0.14
Total Score			60.85	Sensitive	83.63	Moderate	110.06	Tolerant

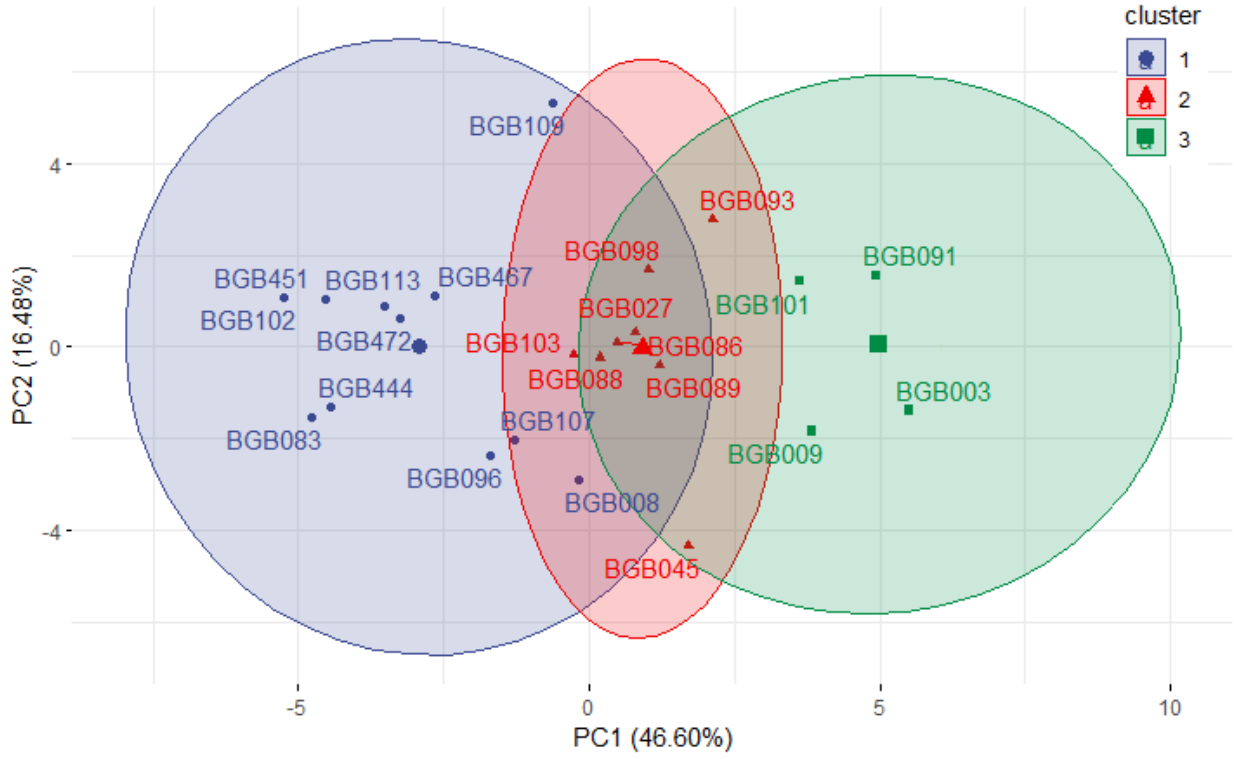

Figure 15: Cluster Plot depicting relationship between 24 potato (*Solanum* sect. *Petota*, Solanaceae) genotypes from Embrapa Potato Genebank under control and heat stress conditions.

3.5. Discussion

Potato wild relatives represent a fundamental source of genetic variability (Jansky et al 2000). Besides evaluating the wild potatoes, this is the first screening comparing the wild germplasm to dihaploid for their response to heat stress conditions. Evaluating genetic diversity in photosynthetic traits can help to improve our understanding of adaptation factors while also providing a wealth of resource diversity that can be explored for industrial or agronomic applications, because photosynthetic traits can aid adaptation to changing environmental conditions, as HS during growth and development can greatly damage the potato crop yield.

In this study, we have demonstrated significant genotypic variation among potato germplasm in specialized growth chambers in terms of SPAD values, net photosynthesis rate, stomatal conductance, transpiration rates, water use efficiency, intrinsic water use efficiency, intracellular CO_2 assimilation of leaves, effective photochemical yield, non-photochemical yield, coefficient of photochemical yield, electron transport rate and maximum photochemical quantum yield of photosystem II. Despite having significant intracultivar and interspecies significant variations, our results established that overall, heat stress decreased stomatal conductance, transpiration rate, intracellular CO_2 assimilation and maximum photochemical quantum yield, whereas increased the SPAD values with increased electron transport rate and net photosynthesis rate, water use efficiency and intrinsic water use efficiency in the studied potato germplasm (Figure 6).

We measured leaf chlorophyll content based on the directly proportional SPAD values ($R^2 = 0.80$) to the absolute chlorophyll content (VILLA E VILA *et al.*, 2022). Our findings show that under heat stress conditions, the leaf chlorophyll content, as measured by SPAD values, increased in most of the genotypes of the observed species. This trait was consistent across three measurements (14, 28 and 42 days after starting the heat stress treatment). These observations of increased SPAD values in heat stressed plants, although repeatable and consistent with the leaf color and morphology of potato plants, are contrary to the findings of reduced SPAD values in wheat (*Triticum aestivum*) and rice (*Oryza sativa*) plants under heat stress (BALOUCHI, 2010; QI-HUA *et al.*, 2013; TIWARI *et al.*, 2017). Our results of 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 *Solanum chacoense* (REYNOLDS; EWING; OWENS, 1990). However, SPAD results from this study were in accordance with the findings of (TANG *et al.*, 2018). 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 must be used to investigate genetic diversity.

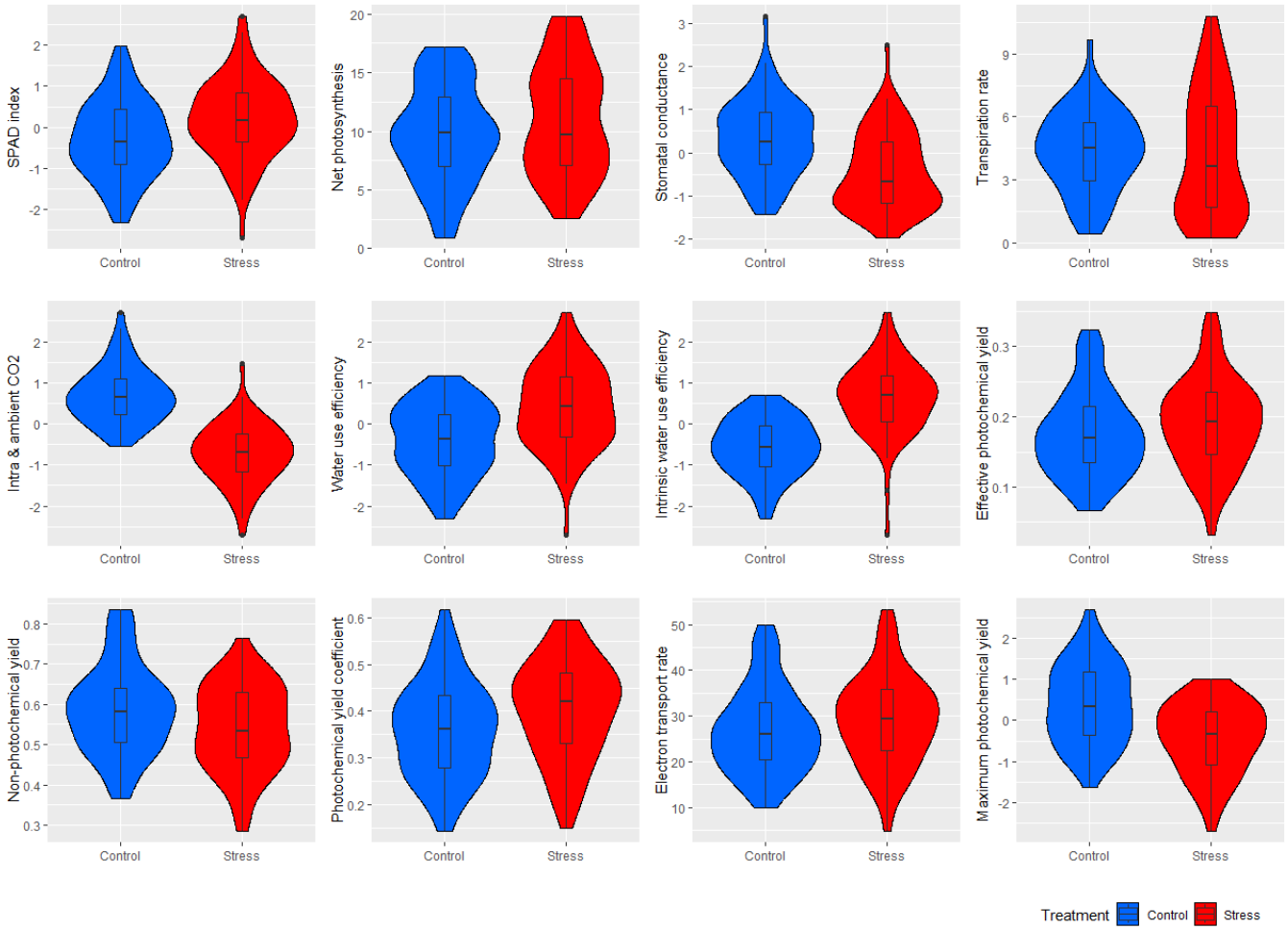

Figure 16: Comparison of physiological traits among 24 genotypes of potato (*Solanum* sect. *Petota*, Solanaceae) under control (CT) and heat stressed (HS) conditions.

During heat stress, the photosynthesis reduction is linked to reduced antenna pigments in chlorophyll content (CAMEJO *et al.*, 2006; HERDE *et al.*, 1999). 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; XU *et al.*, 2006). On the other hand, in our study there is no significant reduction in chlorophyll content in the scale of SPAD value (**Figure 16**) which enables the potato germplasm to withstand heat stress and increase the photosynthesis rate (**Supplementary Table S1**). The higher rate of net photosynthesis of studied germplasm in heat stress temperature was explained by (HAVAUX, 1993) and (WOLF *et al.*, 1990) and proved the existence of adaptive process.

These results also indicate the significant differences of the wild potato germplasm against abiotic stress, specifically heat stress (**Table 2.**). Although, these results do not support the findings in other solanaceous crops such as tomato reported by (ZHOU *et al.*, 2015), the reduction in net photosynthesis rate indicating that the chloroplasts suffered more severe damage and suggested that heat stress negatively affected the photosynthesis and carbohydrate accumulation by decreasing the leaf pigment contents and damaging the leaf ultrastructure. The same phenomena of inhibition of carbohydrate translocation and partitioning at other plant organs was also reported in cereals such as wheat by (SHANMUGAM *et al.*, 2013; WAHID *et al.*, 2007). 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 regulation is a vital protective mechanism for heat tolerance as indicated by plant changes in their morphology by reducing the stomatal number and conductance to avoid water loss by evapotranspiration (GOUFO *et al.*, 2017; SICHER; TIMLIN; BAILEY, 2012). Stomatal conductance plays an important role in increasing 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 during the heat stress (**Figure 16**). Stomatal responses to heat depended on species, however, and paradoxically, two accessions (BGB003, BGB109) opened stomata and significantly increased Gs (**Supplementary Table S1**). Stomatal closure during heatwaves follows stomatal optimization theory (COWAN; FARQUHAR; JENNINGS, 1977)), whereas sacrificing additional water loss under high temperature conditions (e.g., heatwaves) contradicts the current stomatal behavior theory (DAMOUR *et al.*, 2010; LU *et al.*, 2020; SPERRY *et al.*, 2017).

High temperatures resulted in a significant decrease of stomatal resistance (**Figure. 16**). The low stomatal resistance at high temperature may enhance the photosynthetic system in two ways: (a) through increasing CO_2, transport to the substomatal cavities and (b) through cooling the leaves by latent heat due to higher transpiration rates (WOLF *et al.*, 1990). In this study, 50% of used accessions followed the second mechanism of increased transpiration rate to cope with the increased temperature treatment (**Supplementary Table S1**). Other remaining genotypes (BGB003, BGB008, BGB009, BGB027, BGB045, BGB083, BGB086, BGB088, BGB089, BGB091, BGB093, BGB096, BGB098, BGB101, BGB102, BGB103, BGB107, BGB109) showed an increase in transpiration rates (Tr) used in this experiment at the different days after stress measurement under high temperature conditions. This agrees with the findings of Berry and Bjorkman (1980) who reported that transpiration is temperature dependent. This behavior is also in agreement with Nkansah and Ito (1994, 1995), who observed an increase in

transpiration rate under HS in tomato genotypes. Overall, *Solanum chacoense* recorded a decrease in transpiration rate, which shows the intraspecies differences between genotypes in transpiration may be due to their differences in morphology (**Figure 12**).

The concentration of CO2 in the atmosphere is directly related to rising temperature, as predicted by various models (IPCC 2022), and it is also assumed in various growth chamber experiments. According to various studies, rising temperatures will negate the positive effects of CO2 on plant growth (Allen et al., 2003; Horie et al., 2000). In general, rising temperatures cause an increase in WUE and iWUE, but this increase is not always directly 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). Through three sensitive processes, the positive effects of increased ambient CO2 diminish as the temperature rises above the optimum temperature for the species. Firstly, under stress, plants can decrease transpiration due to a decline in Gs, as has been the general rule (Eamus & Jarvis, 1989; Mott, 1990) which is also shown by the many of the used genotypes (BGB045, BGB083, BGB096, BGB102, BGB103, BGB107, BGB113, BGB444, BGB451, BGB467, BGB472) although instances of 'abnormal' stomatal responses have been reported (Eamus & Wilson, 1984) as also shown by BGB003 and BGB101. Second, stress can increase Pn, which was shown in this study as a general response of potato species. However, in numerous research on other agricultural plants, Pn was shown to be lower, unchanged, or only slightly higher than in control plants (Reekie & Bazzaz, 1989; Oberbauer et al., 1985). Third, combining the two can result in improved WUE. As a result, an increase in WUE and iWUE was seen in all genotypes under heat stress conditions in our study by following both processes. Figure 3B also revealed a negative significant link between Pn, E, Gs, Ci/Ca, ETR, YII, qp and can be regarded as modulators that may influence WUE, iWUE which is in accordance with the findings of Condon *et al* (2002).

Because Ci/Ca varies throughout the day, it is necessary to understand the major driving factors that Pn and Gs can cause on Ci/Ca variations (TAN *et al.*, 2017). A high Pn value indicates rapid CO_2 consumption, while a high Gs value indicates less resistance to the entry of ambient CO_2 into intercellular spaces. As a result, high Pn combined with low Gs resulted in low Ci/Ca (COWLING; SAGE, 1998). All genotypes in this study followed the same trend under HS and CT conditions (**Supplementary Table S1**). So, under abiotic stresses such as heat stress, these findings are consistent with those reported by several authors (CAMEJO *et al.*, 2005; KITAO *et al.*, 2003; OLIVEIRA *et al.*, 2022; SAGE, 1994; YU *et al.*, 2014).

Heat tolerance is influenced by the plant ability to cool the leaf by increasing transpiration at high temperatures (CAMEJO *et al.*, 2006; SHARMA *et al.*, 2014). The ability of the tolerant genotypes to maintain high rates of photosynthesis under heat stress created a demand for higher stomatal conductance, which resulted in better evaporative cooling as compared to the heat-sensitive germplasm, as evidenced by lower values of Pn, Gs, E, and Ci/Ca. (**Supplemental Table S1**) for accessions in the heat stress growth chambers.

There was also a strong positive relationship observed in current studies between traits measured with the Li-Cor photosynthetic system (Pn, Gs, E, Ci/Ca.). These findings are supported by Ji et al (2022), who discovered an association of these traits while studying *Paeonia suffruticosa* under high temperature stress. Brodribb and Holbrook (2003) discovered that stomatal closure is closely related to leaf physiological traits during stress. Sharma et al (2015) reported a correlation between net photosynthesis, total chlorophyll, stomatal conductance, and transpiration rates in cereals such as wheat under HS. Despite an efficient photosynthetic response to elevated temperature in our studies (**Figure 16**), indicating an acclimatization of the photosynthetic apparatus (HAVAUX, 1995). High temperatures decrease the time of normal plant growth, leading to smaller organs (Bita and Gerats, 2013). According to Berry and Bjorkman, (1980) and Wahid et al (2007), the primary damage imposed when the temperature exceeds the optimal for photosynthesis is the loss of stability and the disorganization of membranes, which affects the stability of the photosynthetic apparatus. Thus, photosynthesis and respiration, being dependent on electron transport activity and membrane associated enzymes, are reduced when the functional integrity of the chloroplasts and mitochondrial membranes are affected.

Potato plants are subjected to daily abiotic stresses that have a negative impact on their photosynthetic apparatus. Indeed, heat stress, along with other abiotic stresses (high sunlight, water or mineral scarcity, low temperature, heavy metal toxicity, and air pollution), can determine when the light absorbed by chlorophyll pigments becomes excessive for the needs of photosynthetic machinery (MURATA *et al.*, 2007). The first hypothesis on the effects of environmental stresses on PSII activity proposed that stressors accelerated PSII photoinhibition (ADIR *et al.*, 2003; BJÖRKMAN; POWLES, 1984; MELIS, 1999). This hypothesis has recently been challenged, with many researchers demonstrating that the PSII repair mechanism is more sensitive to environmental stresses than the photodamage process itself (ALLAKHVERDIEV; MURATA, 2004; KANGASJÄRVI *et al.*, 2014; NISHIYAMA; ALLAKHVERDIEV; MURATA, 2011; NISHIYAMA; MURATA, 2014; TAKAHASHI; MURATA, 2008). 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 (NISHIYAMA; ALLAKHVERDIEV; MURATA, 2006). 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; 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; CHOW; ANDERSON, 1992). Fv/Fm values typically range between 0.75 and 0.85, and this ratio is proportional to photochemistry's quantum yield (KITAJIMA; BUTLER, 1975).

BGB003 accession of *S. commersonii*, BGB093 of *S. tuberosum* 2x, and five of *S. chacoense* (BGB086, BGB101, BGB109, BGB467, BGB472) in the study germplasm fall within the above-mentioned ratios under heat stress. A decrease in this ratio is thought to be a good indicator of photoinhibition (ÖQUIST; CHOW; ANDERSON, 1992). As shown by 60% of used 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; 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; PARK *et al.*, 1996).

Although the Fv/Fm ratio was estimated in the dark-adapted condition, it is critical to detect PSII photoinhibition through inducing a strong saturating light pulse called quenching analysis (BOLHÀR-NORDENKAMPF; ÖQUIST, 1993). By quenching analysis, it is possible to determine the contributions of photochemical and non-photochemical processes separately (BAKER, 2008; BUTLER, 1978; SCHREIBER *et al.*, 1995). The decrease in fluorescence due to photochemistry, i.e., using excitation energy within photosystem II (PSII) to drive electron transport from the reaction center chlorophyll of PSII (P680) to primary quinone acceptor of PSII (Q_A), is named photochemical quenching. Whereas nonphotochemical quenching occurs when there is an increase in the rate at which excitation energy within PSII is lost as heat (HORTON; WENTWORTH; RUBAN, 2005; LOGAN *et al.*, 2014; MULLER; LI; NIYOGI, 2001; NIYOGI, 2004; RUBAN; MURCHIE, 2012).

Concerning photochemistry, the most useful parameter obtained from quenching

analysis is the measure of the effective photochemical quantum yield of PSII or YII (GENTY; BRIANTAIS; BAKER, 1989). To predict the proportion of open PSII reaction center, coefficient of photochemical quenching, qP is used (MAXWELL; JOHNSON, 2000) which can also be measured by the proportion of closed reaction center with this simple equation 1-qP (HUNER *et al.*, 1996; HUNER; ÖQUIST; SARHAN, 1998). This trait was used as an indicator of the level of photoinhibition occurrence by Ruban and Murchie (2012). 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 act as photoprotection by adjusting the photosynthetic photon density under heat stress conditions (CRITCHLEY; RUSSELL, 1994; GENTY; BRIANTAIS; BAKER, 1989; KRAUSE; 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.

NPQ response changes dynamically under light to maintain a balance between protection and light utilization. From our existing state of information, we can say that regulatory mechanisms of the NPQ play a dynamic role in the acclimation and adaptation of plants to abiotic stresses. In this study, accessions belonging to different species group showed declining trend, in other words, showed higher photoprotection mechanism in having the high values for the NPQ. This proves that genotypes with low values of YII, ETR and qP must have higher values of nonphotochemical quenching. So, there will be a significant negative correlation which is also shown in our studies under stress conditions (**Figure 13B**, **Supplementary Table S2**).

Pearson's correlation results (**Figure 13B, Supplementary Table S2**) under heat stress conditions suggest us that the net photosynthesis rate (Pn) is lead to positive change in all other studied traits except for nonphotochemical quenching. This explains that positive increase in photosynthesis is only possible with low values of NPQ. Although, SPAD chlorophyll values were higher under heat stress condition but has no significant relation with any other studied traits, while a contrary case under control temperature treatment.

The PCA under control and heat stress conditions (**Figure 14, Table 8**) 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. According to ward's clustering method, studied germplasm were divided into three clusters based on their response measured as the studied variable. Under studied conditions (**Figure 15**), cluster 3 contain 18% of total genotypes, which can be considered as tolerant group to heat stress because genotypes belong to this group were higher in net photosynthesis rate (Pn), maintained the stomatal conductivity, leads to higher transpiration rates (E), showed higher instantaneous and intrinsic WUE, intracellular and ambient CO_2 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). Additionally, less heat dissipation was observed in genotypes belonging to cluster 4. Therefore, our data suggest that under heat stress conditions, these genotypes are more efficient in transferring the light excitation energy to CO_2 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 CO_2 assimilation.

3.6. Conclusion

Photosynthetic activity is an important factor to study under heat stress in Solanaceae crops such as potato. Overall, this study found significant variation in HS tolerance among potato genotypes, and several relatively heat stress tolerant and sensitive genotypes were identified based on physiological traits. Combining physiological traits measured by chlorophyll fluorescence analysis with Li-Cor (IRGA) traits would be ideal for genotype screening. This type of non-invasive screening procedure allows recognizing genotypes with overall higher stress tolerance, *i.e.*, at the physiological level; such genotypes can then be used as parental genotypes in breeding programs to develop terminal potato heat tolerant genotypes.

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Supplementary Table 2: Mean performance of wild potato genotypes (*Solanum* sect. *Petota*, Solanaceae) from Embrapa Potato Genebank , 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) under control temperature and heat stress condition.

Genotype	Treatment	SPAD	Pn	Gs	E	Ci/Ca	WUE	iWUE	YII	NPQ	qP	ETR	Fv/Fm
BGB003	Control	35.64±2.01defghijklm	14.87±1.72abc	0.58±0.06a	5.68±0.27a	0.85±0.00abc	2.62±0.31ab	25.63±0.84ab	0.2±0.05ab	0.6±0.11a	0.38±0.06abc	29.79±7.75ab	0.77±0.01abc
BGB008	Control	33.86±1.86fghijklm	11.92±2.2abc	0.55±0.19a	5.2±0.87a	0.85±0.01abc	2.29±0.20ab	24.32±3.69ab	0.14±0.04ab	0.66±0.09a	0.3±0.07abc	21.65±5.61ab	0.75±0.03abcd
BGB009	Control	33.21±1.55fghijklm	15±1.06abc	0.59±0.10a	5.58±0.49a	0.84±0.010abcd	2.72±0.26ab	26.53±3.16ab	0.21±0.06ab	0.63±0.08a	0.45±0.08abc	32.36±8.79ab	0.75±0.01abcde
BGB027	Control	39.83±0.58bcdefghijk	6.76±1.65abc	0.32±0.13a	3.99±1.19a	0.85±0.04ab	1.86±0.36ab	27.93±9.52ab	0.19±0.00ab	0.55±0.04a	0.38±0.02abc	28.62±0.26ab	0.76±0.01abcde
BGB045	Control	32.16±1.51hijklm	10.77±0.33abc	0.53±0.03a	5.63±0.43a	0.88±0.00a	1.94±0.16ab	20.59±0.77b	0.21±0.02ab	0.56±0.02a	0.45±0.04abc	32.74±2.48ab	0.75±0.00abcde
BGB083	Control	30.78±0.28klm	6.61±2.81abc	0.36±0.16a	4.15±1.60a	0.87±0.03a	1.75±0.47ab	26.53±9.40ab	0.13±0.03ab	0.51±0.04a	0.27±0.05bc	19.59±3.90ab	0.73±0.01abcde
BGB086	Control	31.98±1.42hijklm	9.05±0.57abc	0.39±0.13a	4.07±0.47a	0.83±0.01abcd	2.25±0.12ab	27.42±5.94ab	0.19±0.04ab	0.49±0.05a	0.36±0.08abc	29.59±6.83ab	0.77±0.00ab
BGB088	Control	34.79±2.74fghijklm	8.75±0.92abc	0.4±0.21a	4.07±1.12a	0.82±0.06abc	2.44±0.64ab	34.78±14.07ab	0.15±0.04ab	0.5±0.07a	0.29±0.07abc	22.82±6.87ab	0.74±0.01abcde
BGB089	Control	35.29±3.76fghijklm	9.86±1.57abc	0.46±0.25a	4.53±1.28a	0.83±0.04abcd	2.31±0.24ab	31.5±9.14ab	0.2±0.05ab	0.56±0.02a	0.43±0.08abc	31.17±7.68ab	0.74±0.02abcde
BGB091	Control	41±2.84bcdefghij	14.09±2.16abc	0.44±0.08a	5±0.35a	0.82±0.00abcd	2.78±0.27ab	32.24±1.39ab	0.23±0.04ab	0.54±0.09a	0.46±0.03abc	34.93±5.94ab	0.74±0.02abcde
BGB093	Control	35.71±3.63efghijklm	8.36±3.49abc	0.4±0.32a	3.7±2.13a	0.76±0.08abcd	2.81±0.69ab	48.94±19.69ab	0.24±0.03ab	0.48±0.04a	0.46±0.05abc	36.55±5.15ab	0.76±0.02abcd
BGB096	Control	30.64±1.09m	12.9±1.67abc	0.46±0.12a	5.27±0.89a	0.83±0.03abcd	2.55±0.47ab	30.64±6.77ab	0.15±0.01ab	0.74±0.05a	0.37±0.03abc	23.39±1.40ab	0.73±0.00bcde
BGB098	Control	35.53±1.69defghijklm	7.07±3.61abc	0.31±0.19a	3.58±1.79a	0.8±0.06abcd	2.15±0.27ab	35.37±11.57ab	0.18±0.03ab	0.54±0.02a	0.37±0.04abc	28.1±4.79ab	0.75±0.02abcde
BGB101	Control	43.61±1.69abcdef	13.27±1.66abc	0.41±0.05a	4.85±0.41a	0.82±0.01abcd	2.75±0.35ab	32.23±2.10ab	0.21±0.02ab	0.59±0.05a	0.41±0.03abc	31.59±3.91ab	0.76±0.01abc
BGB102	Control	32.13±2.65ijklm	3.99±2.15c	0.12±0.06a	2.16±0.98a	0.82±0.01abcd	1.67±0.20b	32.23±1.37ab	0.14±0.03ab	0.64±0.09a	0.31±0.05abc	22.02±3.99ab	0.75±0.01abcde
BGB103	Control	42.17±3.59abcdefg	9.4±2.47abc	0.38±0.05a	4.64±0.08a	0.86±0.02ab	2.01±0.50ab	24.1±4.22b	0.2±0.05ab	0.6±0.05a	0.4±0.08abc	30.44±8.42ab	0.76±0.01abcd
BGB107	Control	39.37±2.23bcdefghijklm	10.45±0.31abc	0.72±0.48a	5.55±2.10a	0.84±0.07abc	2.41±0.70ab	32.82±14.06ab	0.14±0.02ab	0.69±0.03a	0.3±0.03abc	20.87±3.20ab	0.76±0.01abcd
BGB109	Control	46.8±2.76abcd	7.27±4.43abc	0.1±0.05a	3.25±2.06a	0.81±0.04abcd	2.29±0.11ab	60.96±11.90ab	0.19±0.02ab	0.62±0.01a	0.41±0.03abc	29.31±2.88ab	0.75±0.01abcde
BGB113	Control	40.97±3.33bcdefghi	11.16±2.19abc	0.36±0.14a	4.36±1.01a	0.8±0.03abcd	2.66±0.43ab	36.54±8.30ab	0.17±0.04ab	0.51±0.05a	0.34±0.05abc	26.37±5.70ab	0.75±0.01abcde
BGB444	Control	30.77±2.14m	7.95±1.53abc	0.31±0.09a	4.07±0.87a	0.84±0.02abcd	1.98±0.22ab	27.81±3.63ab	0.14±0.02ab	0.58±0.03a	0.3±0.05abc	21.91±3.54ab	0.75±0.01abcde
BGB451	Control	31.92±0.13ghijklm	7.7±1.31abc	0.26±0.12a	3.55±1.23a	0.77±0.08abcd	2.67±0.80ab	45.12±19.46ab	0.14±0.05ab	0.58±0.08a	0.29±0.09abc	21.35±7.51ab	0.74±0.01abcde
BGB467	Control	34.13±2.16fghijklm	7.52±1.36abc	0.29±0.14a	3.72±1.22a	0.82±0.04abcd	2.2±0.28ab	35.28±9.04ab	0.14±0.03ab	0.64±0.10a	0.27±0.05bc	20.88±5.12ab	0.78±0.00a
BGB472	Control	32.91±2.4ghijklm	8.14±2.12abc	0.28±0.11a	3.82±0.98a	0.83±0.03abcd	2.19±0.46ab	32.92±8.00ab	0.15±0.03ab	0.68±0.07a	0.31±0.06abc	22.24±4.87ab	0.76±0.01abcd
BEL	Control	41.49±2.7bcdefghi	14.38±1.68abc	0.75±0.27a	5.59±1.09a	0.82±0.04abcd	2.68±0.37ab	22.69±4.88b	0.25±0.06ab	0.58±0.09a	0.51±0.09abc	39.05±8.59ab	0.76±0.01abcd
Mean	**CT**	**36.11±2.11a**	**9.89±1.87a**	**0.41±0.15a**	**4.42±1.04a**	**0.83±0.032a**	**2.33±0.37a**	**32.30±7.62a**	**0.18±0.03a**	**0.58±0.06a**	**0.37±0.06a**	**27.39±5.22a**	**0.75±0.01a**
BGB003	Stress	35.06±1.85fghijklm	16.63±1.47ab	0.66±0.32a	7.93±1.53a	0.78±0.07abcd	2.18±0.22ab	38.92±15.17ab	0.27±0.03ab	0.47±0.03a	0.52±0.04ab	41.12±4.02ab	0.75±0.00abcde

BGB008	Stress	35.38±3.49fghijklm	12.6±1.98abc	0.32±0.11a	5.4±1.07a	0.6±0.07abcd	2.39±0.15ab	51±17.18ab	0.15±0.05ab	0.63±0.05a	0.33±0.10abc	22.4±7.68ab	0.71±0.03bcde
BGB009	Stress	31.46±1.56jklm	15.9±0.98abc	0.34±0.08a	7.05±0.85a	0.71±0.06abcd	2.29±0.15ab	53.34±13.90ab	0.22±0.03ab	0.56±0.05a	0.47±0.03abc	34.43±3.84ab	0.74±0.01abcde
BGB027	Stress	42.02±1.82abcdefgh	10.76±1.47abc	0.19±0.10a	5.05±1.62a	0.6±0.10abcd	2.36±0.35ab	78.55±21.93ab	0.23±0.00ab	0.48±0.06a	0.47±0.03abc	35.12±0.60ab	0.73±0.02abcde
BGB045	Stress	22.47±11.23lm	9.03±4.52abc	0.18±0.10a	3.83±2.04a	0.47±0.24abcd	1.65±0.88ab	37.91±22.02ab	0.18±0.09ab	0.26±0.14a	0.34±0.17abc	27.8±14.49ab	0.49±0.25cde
BGB083	Stress	36.77±0.71cdefghijklm	5.41±0.72bc	0.03±0.010a	1.09±0.35a	0.08±0.15cd	5.56±0.99ab	211.14±38.68ab	0.16±0.01ab	0.58±0.06a	0.35±0.04abc	24.37±2.14ab	0.73±0.00abcde
BGB086	Stress	34.9±0.84fghijklm	11.2±1.23abc	0.23±0.06a	4.65±0.68a	0.66±0.08abcd	2.44±0.11ab	58.04±16.34ab	0.17±0.03ab	0.54±0.05a	0.34±0.06abc	25.82±4.27ab	0.76±0.00abcd
BGB088	Stress	38.9±1.57bcdefghijklm	12.36±2.02abc	0.22±0.08a	5.23±1.57a	0.62±0.11abcd	2.73±0.62ab	75.05±26.29ab	0.22±0.04ab	0.49±0.06a	0.44±0.07abc	33.17±5.88ab	0.74±0.01abcde
BGB089	Stress	40.13±1.24bcdefghij	11.06±4.17abc	0.27±0.18a	5.2±2.44a	0.59±0.13abcd	2.56±0.47ab	78.88±29.25ab	0.19±0.03ab	0.58±0.05a	0.43±0.07abc	29.04±5.14ab	0.72±0.00cde
BGB091	Stress	48.17±0.74abc	17.53±1.37a	0.37±0.07a	7.62±0.71a	0.73±0.02abcd	2.31±0.04ab	49.34±6.69ab	0.25±0.05ab	0.45±0.09a	0.51±0.04abc	38.76±7.28ab	0.72±0.02bcde
BGB093	Stress	42.92±1.88abcdef	12.01±3.86abc	0.29±0.19a	5.44±2.76a	0.41±0.33abcd	4.19±2.19ab	129.67±84.14ab	0.23±0.02ab	0.47±0.03a	0.46±0.02abc	35.99±2.50ab	0.75±0.00abcde
BGB096	Stress	32.56±1.48ghijklm	8.37±1.13abc	0.09±0.02a	2.94±0.66a	0.51±0.06abcd	3±0.51ab	101.37±15.52ab	0.16±0.02ab	0.68±0.05a	0.4±0.06abc	24.11±3.34ab	0.71±0.00cde
BGB098	Stress	37.97±0.53bcdefghijklm	12.32±2.29abc	0.22±0.09a	4.97±1.57a	0.59±0.14abcd	2.81±0.53ab	83.92±35.07ab	0.26±0.04ab	0.47±0.09a	0.5±0.05abc	39.41±5.87ab	0.74±0.00abcde
BGB101	Stress	42.94±0.91abcdef	17.07±2.03ab	0.41±0.15a	7.23±1.57a	0.68±0.10abcd	2.49±0.31ab	59.32±24.20ab	0.21±0.01ab	0.54±0.06a	0.44±0.02abc	32.95±2.12ab	0.75±0.00abcde
BGB102	Stress	34.5±0.93fghijklm	6.4±1.96abc	0.05±0.03a	1.94±0.93a	0.3±0.14abcd	3.89±0.89ab	148.38±32.69ab	0.12±0.04ab	0.6±0.05a	0.37±0.01abc	18.05±6.63ab	0.72±0.02bcde
BGB103	Stress	40.16±3.59bcdefghij	8.96±1.97abc	0.09±0.04a	2.86±1.08a	0.32±0.27abcd	4.31±1.80ab	147.8±66.82ab	0.2±0.02ab	0.48±0.04a	0.41±0.03abc	31.34±2.56ab	0.74±0.01abcde
BGB107	Stress	40±1.25bcdefghijkl	11.3±2.78abc	0.15±0.06a	4.11±1.48a	0.52±0.12abcd	3.28±0.76ab	99±31.78ab	0.14±0.02ab	0.6±0.03a	0.35±0.02abc	22.06±3.79ab	0.68±0.04de
BGB109	Stress	53.74±1.89a	10.61±4.94abc	0.24±0.21a	4.04±2.85a	-0.1±0.66bcd	6.76±3.79ab	300.54±212.6ab	0.22±0.04ab	0.56±0.08a	0.44±0.05abc	34.19±5.78ab	0.76±0.00abcde
BGB113	Stress	46.3±2.18abcde	7.22±1.86abc	0.09±0.06a	1.14±0.32a	-0.05±0.14d	6.57±1.08a	162±66.29ab	0.1±0.01b	0.62±0.03a	0.23±0.02bc	15.03±1.39b	0.68±0.01e
BGB444	Stress	35.16±3.23fghijklm	7.19±1.15abc	0.06±0.02a	1.97±0.57a	0.08±0.27cd	4.35±1.27ab	143.97±46.11ab	0.11±0.01b	0.51±0.10a	0.23±0.03c	16.54±1.77b	0.73±0.01abcde
BGB451	Stress	34.12±2.08fghijklm	6.66±1.21abc	0.03±0.01a	1.08±0.23a	-0.08±0.07d	6.3±0.64ab	245.8±14.52a	0.17±0.01ab	0.63±0.03a	0.4±0.02abc	26.46±1.21ab	0.73±0.01bcde
BGB467	Stress	41.17±3.58bcdefghi	9.7±2.89abc	0.13±0.09a	3.22±1.78a	0.29±0.26abcd	4.51±1.57ab	154.33±64.19ab	0.17±0.03ab	0.53±0.02a	0.34±0.06abc	25.4±4.77ab	0.75±0.01abcde
BGB472	Stress	38.17±3.35bcdefghijklm	7.99±0.76abc	0.04±0.00a	1.64±0.15a	0.2±0.01bcd	4.89±0.36ab	179.49±2.25ab	0.18±0.03ab	0.53±0.07a	0.35±0.06abc	27.03±5.20ab	0.76±0.00abcd
BEL	Stress	49.23±1.83ab	16.5±1.16ab	0.55±0.29a	7.48±1.32a	0.68±0.11abcd	2.28±0.22ab	46.08±16.36ab	0.29±0.02a	0.49±0.06a	0.57±0.02a	44.82±2.49a	0.75±0.00abcde
Mean	**HS**	**38.925±2.24b**	**11.03±2.08b**	**0.22±0.09875b**	**4.30±1.26a**	**0.43±0.16b**	**3.59±0.83b**	**113.91±38.33b**	**0.19±0.03b**	**0.53±0.06b**	**0.40±0.05bc**	**29.39±4.37b**	**0.72±0.02b**

Supplementary Table 3: Correlation between different variables of chlorophyll fluorescence and photosynthesis traits in 24 potato (*Solanum* sect. *Petota*, Solanaceae) genotypes leaves from Embrapa Potato Genebank under control treatment (CT) 14-27°C, and heat stress (HS) 24-34°C. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Traits	SPAD	Pn	Gs	E	C*i*/C*a*	WUE	iWUE	YII	NPQ	qP	ETR	Fv/Fm
SPAD	CT	0.239	0.024	0.117	-0.202	0.381	0.300	0.471*	-0.102	0.438*	0.477*	0.224
p-value	HS	0.261	0.913	0.587	0.343	0.066	0.154	0.020	0.637	0.032	0.019	0.293
Pn	0.282	CT	0.842***	0.879***	-0.030	0.788***	-0.316	0.498*	0.154	0.545**	0.499*	-0.015
p-value	0.182	HS	0.000	0.000	0.890	0.000	0.133	0.013	0.473	0.006	0.013	0.944
Gs	0.224	0.959***	CT	0.954***	0.343	0.484**	-0.716***	0.360	0.088	0.382	0.359	-0.005
p-value	0.293	0.000	HS	0.000	0.101	0.017	0.000	0.084	0.682	0.065	0.085	0.980
E	0.217	0.973***	0.972***	CT	0.381	0.511*	-0.604**	0.349	0.188	0.412*	0.349	-0.086
p-value	0.308	0.000	0.000	HS	0.067	0.011	0.002	0.094	0.380	0.046	0.095	0.688
Ci/Ca	0.003	0.864***	0.907***	0.926***	CT	-0.502*	-0.735***	-0.152	0.060	-0.112	-0.155	-0.216
p-value	0.988	0.000	0.000	0.000	HS	0.013	0.000	0.479	0.779	0.603	0.469	0.310
WUE	0.165	-0.708***	-0.758***	-0.817***	-0.877***	CT	0.205	0.493*	-0.035	0.481*	0.494*	0.121
p-value	0.441	0.000	0.000	0.000	0.000	HS	0.336	0.014	0.871	0.017	0.014	0.575
iWUE	0.097	-0.705***	-0.799***	-0.777***	-0.825***	0.917***	CT	-0.092	0.025	-0.086	-0.089	0.037
p-value	0.653	0.000	0.000	0.000	0.000	0.000	HS	0.668	0.906	0.688	0.678	0.862
Y(II)	0.349	0.748***	0.714***	0.768***	0.696***	-0.572**	-0.476*	CT	-0.372	0.964***	1.000***	0.113
p-value	0.095	0.000	0.000	0.000	0.000	0.003	0.019	HS	0.073	0.000	0.000	0.600
NPQ	-0.013	-0.290	-0.283	-0.348	-0.337	0.586**	0.554**	-0.483*	CT	-0.182	-0.370	0.011
p-value	0.952	0.169	0.180	0.096	0.107	0.003	0.005	0.017	HS	0.394	0.075	0.959
qP	0.302	0.713***	0.675***	0.746***	0.701***	-0.537**	-0.400	0.938***	-0.268	CT	0.964***	-0.052
p-value	0.151	0.000	0.000	0.000	0.000	0.007	0.053	0.000	0.205	HS	0.000	0.811
ETR	0.349	0.748***	0.714***	0.768***	0.695***	-0.572**	-0.476*	1.000***	-0.484*	0.938***	CT	0.115
p-value	0.095	0.000	0.000	0.000	0.000	0.003	0.019	0.000	0.017	0.000	HS	0.593
Fv/Fm	0.196	0.324	0.319	0.314	0.292	-0.123	-0.035	0.537**	-0.236	0.399	0.535**	CT
p-value	0.360	0.123	0.129	0.136	0.167	0.567	0.872	0.007	0.268	0.054	0.007	HS

Supplementary Table 4: Descriptive statistics of physiological traits of 24 potato (*Solanum* sect. *Petota*, Solanaceae) genotypes for different clusters under control (CT) and heat stress (HS) condition.

Cluster-I	Category mean	Mean	*p.value*	Cluster-III	Category mean	Mean	*p.value*
WUE-HS	1.21	0.37	0.00	**Pn-HS**	16.73	11.03	0.00
iWUE-HS	1.22	0.59	0.00	**Gs-HS**	0.59	-0.38	0.00
WUE-CT	-0.65	-0.37	0.05	**Pn-CT**	14.32	9.88	0.00
Gs-CT	-0.01	0.38	0.02	**E-HS**	7.46	4.30	0.00
Pn-CT	7.58	9.88	0.02	**WUE-CT**	0.19	-0.37	0.00
E-CT	3.69	4.42	0.01	**C*i*/C*a*-HS**	-0.05	-0.70	0.00
qP-HS	0.32	0.40	0.00	**Y(II)-HS**	0.25	0.19	0.00
Y(II)-CT	0.14	0.18	0.00	**ETR-HS**	38.42	29.39	0.00
ETR-CT	22.05	27.39	0.00	**qP-HS**	0.50	0.40	0.00
Y(II)-HS	0.14	0.19	0.00	**ETR-CT**	33.55	27.39	0.01
ETR-HS	21.84	29.39	0.00	**Y(II)-CT**	0.22	0.18	0.01
qP-CT	0.30	0.37	0.00	**qP-CT**	0.44	0.37	0.01
Pn-HS	7.23	11.03	0.00	**Gs-CT**	0.92	0.38	0.01
Gs-HS	-1.09	-0.38	0.00	**E-CT**	5.34	4.42	0.01
C*i*/C*a*-HS	-1.30	-0.70	0.00	**WUE-HS**	-0.25	0.37	0.03
E-HS	1.73	4.30	0.00	**iWUE-HS**	-0.02	0.59	0.01

CHAPTER IV

4. Genetic parameters and responses associated with high temperature effects evaluation in potato wild relatives[4]

4.1. Abstract

Crop wild relatives (CWRs) have significantly been used in potato (*Solanum tuberosum*, Solanaceae) breeding. Hence, introgression breeding may help in coping with the challenges posed by climate change. We used 21 accessions from Embrapa Potato Genebank, 12 belongs to wild specie *Solanum chacoense* and 8 from *S. commersonii* and 1 *S. tuberosum* commercial cultivar for their tolerance to two different temperature conditions CT as control temperature (14-24ºC) and HS as heat stress (24-37ºC). The evaluation was based on gas exchange (Pn, Gs and Tr), chlorophyll fluorescence analysis (YII, NPQ, Fv/Fm), chlorophyll A, B and carotenoid content, total water % and tuber yield related traits (FTW and DMC%) and measured after 1DAS (Days after stress), 15DAS and 35DAS. Significant differences were observed between *Solanum* wild genotypes for all types of stresses. Cluster analysis based on principle component analysis grouped the wild genotypes into different clusters based on their properties assessed during stress conditions and cluster scoring from communality percentages classify the genotypes with higher score as tolerant genotypes under heat stress conditions. Among the wild species, tolerance to all stresses was great in *S. chacoense*., to all stresses except heat in S. acaule and to heat and cold in S. commersonii. The correlations among the accessed traits were found here significant for heat stress conditions. Mixed model methodology helps us ranking the genotypes based on measured variables according to their true genotypic values for both temperature conditions and after each measurement of days after applied stress.

Keywords: *Solanum tuberosum;* Crop wild relatives; Heat stress; Mixed models.

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4.2. Introduction

Temperature influences the internal biological reactions that control growth and development, influences photosynthesis and respiration, establishes crop cultivation boundaries (Geange et al., 2021), and is a worrying climate change factor (e.g., water stress, salinity, ozone, and CO_2) that will affect crop yields (Jin et al., 2017; Piao et al., 2017). Temperature increases are projected to range from 2.1°C to 5.7°C globally by the end of the 21st century (IPCC, 2021) and from 2°C to 3.8°C in the Midwest of the United States (Jin et al., 2017). Temperature variability is a cause for concern because most cultivated species have lower heat tolerance limits (Geange et al., 2021). As a result, crops must be bred to perform well at higher temperatures.

Plant heat stress (HS) is the exposure of a species to air temperatures above its optimum physiological range for a period ranging from minutes to months in order to maintain maximum productivity, resulting in acute and chronic effects on overall plant performance. It is not unusual for HS to refer to heat shock, heat waves, and climate change. Heat shock can occur once at any growth stage, lasting from minutes to hours, and is frequently observed in molecular studies; heat waves are intermittent stresses that can last from a few hours to days and are typically imposed in experiments on critical growth stages (Siebers et al., 2015); and warming is a general temperature rise that occurs on average over a month (S. Jagadish et al., 2021). The effect on plants is determined by the duration, frequency, and intensity of the specific environment, which has recently become unpredictable and unavoidable. Temperatures above 45°C or 10-15°C above optimal may cause irreversible damage to most species (Panthee & Gotame, 2020; Taiz et al., 2015). HS not only endangers crop systems by decreasing food production, but it also places additional strain on irrigation-dependent zones (Parker et al., 2020a). These effects may eventually lead to food insecurity and social unrest in regions reliant on monoculture and staple foods.

Potato is, a nutritious crop which can be grown in many environments and could be an important food to help with the world's increasing demand for food due to its growing population. However, the yield of potatoes in the tropics and sub-tropics is less than 1/3 compared to the temperate zones (Kooman,1995). Tuber yield and quality of potato reduces with high temperatures, one of the main hurdles for increasing potato yield in warmer areas and seasons. High temperatures decrease the production of crops as they limit plant growth due to factors such as heat stress and/or reduced partitioning of photo assimilates to potatoes. One

important factor for the potato crop is minimal night temperatures as, potato tuberization is decreased when temperatures at night are above 20°C and if they are 25°C and above, no tuberization will occur. Injuries due to high temperature stress may eventually result in growth suppression, starvation, reduced iron flux and might even result in generation of reactive oxygen species and/or toxic substances (Schoffl et al., 1999; Howarth, 2005). After exposure to elevated temperature, heat shock proteins are activated; this is known to be an important environmental adaptive strategy in this regard (Feder and Hoffman, 1999, Janni et al., 2020). The tolerance caused due to exposure to so, results in many benefits, such as better photosynthesis, water use and nutrient use efficiency, assimilate partitioning and stability of cell membranes (Camejo et al., 2005; Ahn and Zimmermann, 2006 and Momcilovic and Ristie, 2007). Due to these adaptations, plants are able to grow and develop under heat stress. Successful attempts have already been made to strengthen heat tolerance through traditional crop breeding methods (Ehlers and Hall, 1998, Camejo et al., 2005, Driedonks et al., 2016). There is a huge amount of variation between and within species, this provides an opportunity to improve the tolerance of crops for heat stress through genetic means. So, keeping the above in view research was performed to study the morphological and physiological parameters with wild potato accessions of diverse nature.

4.3. Material and methods

The experiment follows a randomized complete block design (RCBD) with 2 factors, genotypes (G) and environment conditions designated as control (C) and stress (HS). Tubers from 20 accessions (Table 9) of uniform size were selected and acclimatized and grown in controlled condition as discussed in previous chapters. After uniform growth they were transferred to growth chambers with two replications of each accession. Regular irrigation and pest scouting were performed daily. Data was recorded after 1 day after stress (DAS) application, 15 DAS and 35DAS (**Figure 17**).

Table 8: Germplasm from Embrapa Potato Genebank evaluated for genotypic response under heat stress.

Solanum chacoense	***Solanum commersonii***
BGB094, BGB095, BGB097, BGB099, BGB100, BGB100, BGB104, BGB105, BGB106, BGB108, BGB110, BGB111	BGB001, BGB011, BGB048, BGB055, BGB068, BGB077, BGB453, BGB460

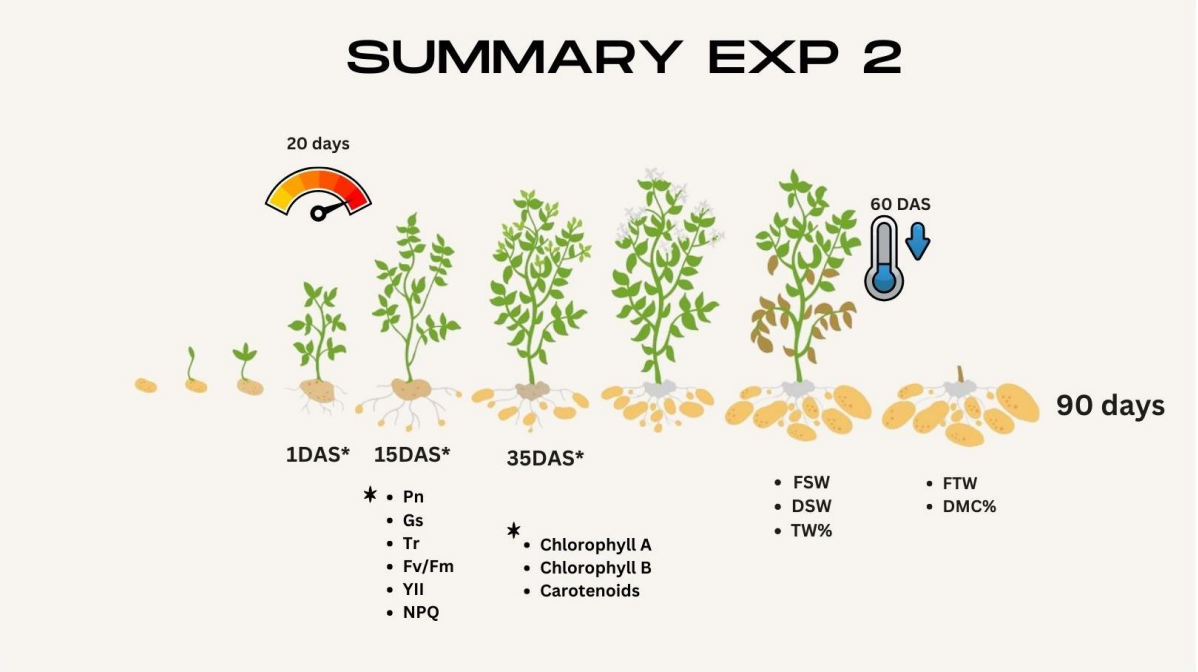

Figure 17: Graphical summary of experiment 2 conducted during December-March 2021.

Two temperature ranges were applied in separate controlled chambers (**Figure 18**). Control Treatment (CT), had a temperature range 14-27°C and for heat stress treatment (HS), temperature range was 24-34°C. Both chambers had controlled photoperiod of 12 hours (7:00 at 19:00h) with light intensity 400 µmol m^{-2} s^{-1} and relative humidity was maintained between 50-60% throughout the experiment.

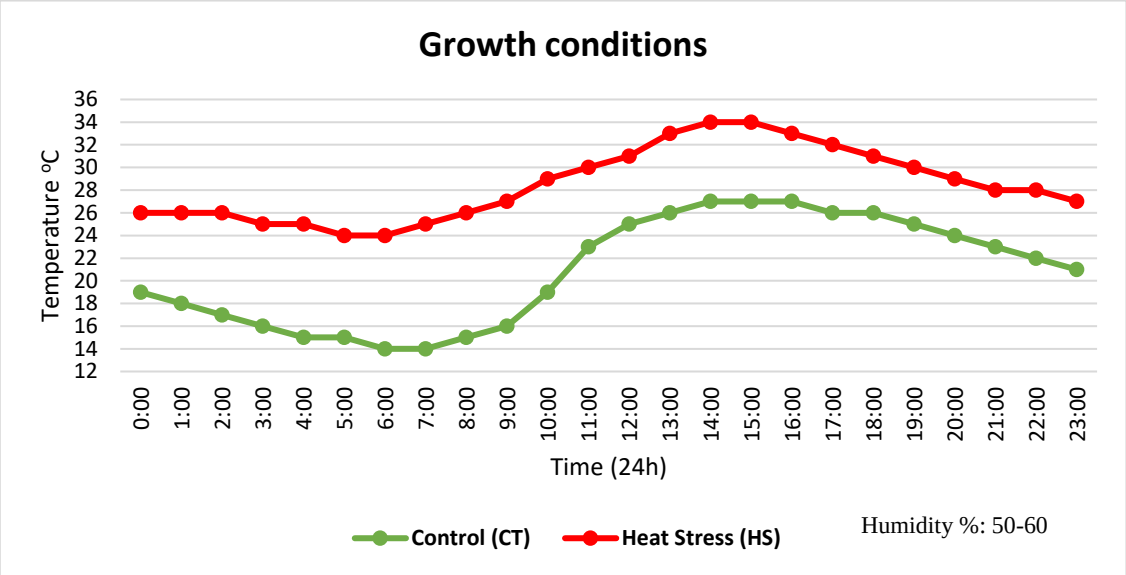

Figure 18: Growth chambers control and heat stress temperature environmental conditions

4.3.1. Chlorophyll fluorescence analysis

Chlorophyll fluorescence was measured using the 4th fully expanded leaf from the top of three plants per genotype and per treatment by using the portable photosynthesis system LI-6400XT

(LI-COR Inc., Lincoln, NE, USA). The middle portion of the leaf was dark adapted for 20 min by using leaf clips (LI-COR Inc., Lincoln, NE, USA) The chlorophyll fluorescence parameters were measured immediately after dark adaptation between 9:00 a.m. and 1:00 p.m. The maximal photochemical efficiency of Photosystem II (PSII) [(Fv/Fm=(Fm− Fo)/Fm)], effective quantum yield of PSII [ΔF/Fm′= (Fm′−F)/Fm′)] and electron transport rate (ETR) were measured at different days after treatment during recovery i.e., 2 days after treatment (2DAT), 7 days after treatment (7DAT) and 15 days after treatment (15DAT) according to methods, as described in Maxwell and Johnson (2000).

4.3.2. Leaf gas Exchange Response

The leaf gas exchange parameters such as, net photosynthetic rate (Pn) and stomatal conductance (Gs) and transpiration rate (Tr) were measured at different days after stress i.e., 1 days after stress (1DAS), 15 days after stress (15DAS) and 35 days after stress (35DAS). The measurements were performed on the fully expanded 4th leaf with two plants per genotype and per treatment between 9:00 a.m. and 1:00 p.m. The portable photosynthesis system LI-6400XT (LI-COR Inc., Lincoln, NE, USA) equipped with an open-flow infrared gas analyzer was used at a steady state (PAR of 100–800 µmol m^{-2} s^{-1}, reference CO_2 concentration of 400 µmol mol^{-1}, air flow rate of 500 µmol s^{-1}. Measurements were performed on the third expanded leaf from the top of the stem on a plant of each genotype previously irrigated and adapted for at least 30 minutes at a temperature of 24°C for plants in the control temperature condition, and 34°C for plants in the stress condition.

4.3.3. Determination of Chlorophyll fluorescence (CF) variables under artificial illumination

Chlorophyll fluorescence analysis were performed using the PAM-2500 fluorometer (Walz Heinz GmbH, Effeltrich, Germany). Before measurements, the plants were dark-adapted inside each growth chamber for at least 30 minutes. The initial fluorescence (Fo) in the open centers of photosystem II (PSII) was determined by measuring light (less than 30 µmol m^{-2} s^{-1}), while the maximum fluorescence (Fm) in closed centers or in a reduced state of the PSII was evaluated after application of a 0.8 second pulse of saturation light (7000 µmol m^{-2} s^{-1}). The maximum quantum efficiency of the PSII (Fv/Fm) was defined as (Fm - Fo)/Fm. The induction curves were made by pulse of saturation light applied every 20 seconds until steady state was reached. During measurements, actinic light (red light) was activated to quantify steady-state chlorophyll fluorescence. In plants in the light-adapted state, Fm' was analyzed by applying a

saturating pulse, while Fo' was assessed by turning off the actinic light for 2 seconds after the saturation pulse and turning on the far-red light. The effective photochemical quantum yield of PSII (Y(II)) was defined as (Fm'- Fs)/Fm' (BAKER, 2008). The most straightforward way of quantifying non photochemical quenching is by measuring the ratio of a change in Fm to the final value of Fm (Bilger and Bjo¨rkman, 1990). NPQ (sometimes referred is linearly related to heat dissipation and lies on a scale 0–infinity). In a typical plant, values might be expected in the range 0.5–3.5 at saturating light intensities. However, this varies markedly between species and on the previous history of the plant.

$$NPQ = (Fm - Fm')/Fm'$$

The response values for Y(II), NPQ, Fv/Fm were calculated by taking the average of the values obtained in light 281, 336, 396 and 461 μmol m^{-2} s^{-1}.

4.3.4. Determination of chlorophyll content

Chlorophyll A, chlorophyll B, and carotenoids were determined as described by Wellburn (1994) with small modifications. The absorption was measured at 480, 649, and 665nm using a microplate reader SpectraMax® M3. Contents of chlorophyll a, chlorophyll b and carotenoid were calculated by the formulas

$Chl_a = (12.19 \times A_{665}) – (3.45 \times A_{649})$

$Chl_b = (21.99 \times A_{649}) – (5.32 \times A_{665})$

$Cart = ((10^3 \times A480) – (2.14 \times Chl_a) – (70.16 \times Chl_b)) \div 220$, respectively.

4.3.5. Total water contents %

At the end of the plant life cycle, arial parts were collected and weighed in grams (g) and collected parts were moved to drying oven. Dry weight measurements were taken after oven-drying at 70°C until a constant weight in grams (g).

Total water (% TW) in shoots was determined as (fresh weight - dry weight) × 100.

4.3.6. Fresh Tuber Weight (FTW)

At the end of the potato plant life cycle, pots were extracted out of the growth chambers and allowed them to dry the substrate and later tubers were collected and fresh tuber weight was calculated in grams (g).

4.3.7. Dry matter content %

DMC% was determined by oven-dry method, with 2 to 3 raw tubers (randomly selected) peeled, chopped, and put in glass petri dishes. Fresh weight of tuber samples was taken by using electronic balance. Later, samples were oven dried at 70ºC for 72h. Dried tuber samples were weighed again. Each genotype was replicated twice (Naeem and Caliskan, 2020).

DMC% was determined by the oven-dried method = Oven dry weight ÷ initial fresh weight × 100

4.3.8. Statistical Model

Collected data were analyzed using the R Program version 4.2.1 in Rstudio IDE version 2022.7.2.576. Data were subjected, when appropriate, to two-way factorial analysis of variance (ANOVA) for treatment and Genotype. Differences between means ($P \leq 0.05$) were separated by Scott Knot's tests for both temperature regimes separately to determine the significance of variation among the different genotypes.

Two statistical models were used to estimates of the variance components and predictions of the genetic values made using the REML/ BLUP (Restricted Maximum Likelihood/Best Linear Unbiased Predicted) procedure. The first model tested (54) considered the genotype × environment interaction, and the second model (21) disregarded the genotype × environment interaction, respectively.

Model 54

$Y = Xr + Zg + Wi + e$

Model 21

$Y = Xr + Zg + e$

where Y is the data vector, *r* is the vector of repetition effects (assumed to be fixed) added to the overall mean, *g* is the vector of genotypic effects (assumed to be random) and *e* is the vector of errors or (random) residuals and *i* is the vector of the effects of the genotype × environment interaction (random). The capital letters represent the incidence matrices for these effects.

4.3.9. Broad sense heritability

The broad-sense heritability of the evaluated characters was calculated using the variance components of the REML (Restricted Maximum Likelihood Method), using the formula cited by Fehr (1987):

where h^2 is the calculated value of heritability in the broad sense, σ2g is the genotypic variance;

σ2e is the variance of the environment.

$H_g^2 = \frac{\sigma_g^2}{\sigma_g^2+\sigma_{g_e}^2+\sigma_e^2}$ broad sense heritability of the G × T interaction

$H_g^2 = \frac{\sigma_g^2}{\sigma_g^2+\sigma_e^2}$ heritability without interaction effect

σ^2_g: genotypic variance among genotypes;

σ^2_{int}: variance of the genotype × environment interaction;

σ^2_e: residual variance;

The heritability values were classified according to Stansfield (1974), where heritability values greater than 0.5 are considered high, values between 0.2 and 0.5 average, and less than 0.2 are considered low heritability.

4.3.10. Genotype selection accuracy

The accuracy estimator was calculated according to the formula proposed by Resende (2002).

$A_g = \sqrt{\frac{\sigma_g^2}{\sigma_g^2+\frac{\sigma_{int}^2}{t}+\frac{\sigma_e^2}{r}}}$ corresponds to accuracy under G × T

$A_g = \sqrt{\frac{\sigma_g^2}{\sigma_g^2+\frac{\sigma_e^2}{r}}}$ corresponds to genotype selection accuracy with not interaction.

r = number of repetitions

t = number of treatments (control and stress i.e., 2)

4.3.11. Genotype Ranking

The genotypes were ordered for each measured variable, according to the estimated genotypic value, based on the model (54 or 21), using the REML/BLUP approach (restricted maximum likelihood/best unbiased linear prediction).

4.3.12. Principal Component Analysis (PCA)

Based on the predicted genotypic value for each genotype, in each, control and stress condition, principal component analysis was performed. Principal component analysis for morphophysiological variables under both CT and HS condition evaluated: 20 genotypes based on 12 variables: Pn, Gs, Tr, YII, NPQ, Fv/Fm, Ca, Cb, Cc, TW%, FTW and DMC for all measured DAS (1, 15, 35). To perform these analyses, the R Studio statistical package Factoextra and FactoMineR were used.

4.3.13. Correlation Analysis

Based on the estimated genotypic value for each genotype, Pearson's correlation analysis, for

all measured variables under CT and HS conditions for 1DAS, 15DAS and 35DAS. To carry out these analyzes the statistical R package "PerformanceAnalytics" V2.0.4 was implemented in R Studio. Correlations were classified as: null (r = 0), weak (0<| r| ≤30), medium (30 < |r| ≤ 60), strong (<60 |r| ≤90), very strong (90< |r| <1) and perfect (|r| = 1) according to Carvalho et al. (2004).

4.3.14. Clustering

The 20 genotypes were clustered into ward's method and using PCA values of traits measured. The numbers of clusters were defined by using inertia gain from ward's method. The distance between and within clusters were also estimated using Euclidean. Finally, clustered genotypes were displayed in colored dendrogram.

Cluster score was calculated as a weighted linear combination of physiological traits in order to designate the cluster based on their tolerance level (*i.e.* summation of weightage multiplied with their respective physiological trait). Cluster score = $\sum_{i=1}^{n} X_i W_i$

Where X*i* is the mean value of the *i*th measured trait in the given cluster and W*i* is the weightage associated with the *i*th trait in the given cluster. Weightage was obtained from PCA analysis of communities. Clusters with the highest score were classified as tolerant.

4.4. Results

4.4.1. Analysis of variance (ANOVA)

The ANOVA for gas exchange, chlorophyll fluorescence traits showed significant (p ≤ 0.05) differences among genotypes and interaction effect measured after 1DAS, 15DAS and 35DAS, while in treatment effect there were some non-significant differences observed. Chlorophyll A, B and carotenoid were found to have non-significant differences except for the situation when measured after 35DAS for treatment effect and 1DAS for genotypic effect and interaction effect in case of carotenoid contents. TW% showed significance for genotypic effect. FTW showed significant effects for treatment and genotype effects while DMC% was also significant for treatment, genotype, and interaction effects (Table10).

Table 9: Summary of the analysis of variance for the variables: net photosynthesis rate (Pn), stomatal conductance rate (Gs), transpiration rate (Tr), effective photochemical quantum yield of PSII (YII), non-photochemical quenching (NPQ) , maximum quantum efficiency of the PSII (Fv/Fm), chlorophyll A (Ca), chlorophyll B (Cb), carotenoid contents (Cc), on the first day after stress 1DAS, 15DAS, 35DAS and total water % (TW%), fresh tuber weight (FTW), dry matter content % (DMC) at the end of the plant life cycle of potato genotypes (*Solanum* sect. *Petota*, Solanaceae) from Embrapa Potato Genebank.

SOV	DAS	Pn	Gs	Tr	YII	NPQ	Fv/Fm	C_a	C_b	C_c	TW%	FTW	DMC
Treatment	1	1.00^{ns}	0.02^{*}	0.07^{ns}	0.00^{*}	0.34^{ns}	0.00^{*}	0.62^{ns}	0.27^{ns}	0.37^{ns}	0.96^{ns}	0.00^{*}	0.03^{*}
	15	0.00^{*}	0.00^{*}	0.71^{ns}	0.30^{ns}	0.00^{*}	0.00^{*}	0.18^{ns}	0.87^{ns}	0.07^{ns}			
	35	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.03^{*}			
Genotype	1	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.59^{ns}	0.74^{ns}	0.01^{*}	0.01^{*}	0.00^{*}	0.00^{*}
	15	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.10^{ns}	0.12^{ns}	0.21^{ns}			
	35	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.12^{ns}	0.18^{ns}	0.15^{ns}			
G x T	1	0.00^{*}	0.00^{*}	0.01^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.13^{ns}	0.49^{ns}	0.02^{*}	0.30^{ns}	0.14^{ns}	0.03^{*}
	15	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.35^{ns}	0.53^{ns}	0.13^{ns}			
	35	0.05^{*}	0.02^{*}	0.04^{*}	0.00^{*}	0.00^{*}	0.00^{*}	0.42^{ns}	0.73^{ns}	0.36^{ns}			

*significant at 5% proabability; ns: non-significant

4.4.2. Restricted maximum likelihood (REML)

The heritability values observed in the present study ranged from 0.002 to 0.97 for the Fv/Fm variables at 35DAS under HS and C_b for interaction effect, respectively. The heritability values obtained a significant reduction in the control when in comparison to HS condition. In the control, the observed values ranged from 0.01 to 0.97, being classified as low to high heritability, for the variables TW% and Fv/Fm-15DAS, respectively. The variables Pn, Gs, Tr, YII, NPQ, Fv/Fm, C_c measured after 1DAS, Pn, Gs, Tr, YII, NPQ, Fv/Fm measured after 15DAS and Gs, Tr, YII, NPQ, Fv/Fm at 35DAS, for morphological traits DMC, FTW showed high heritability in the control, the other variables showed heritability estimates classified as medium to low magnitude. In the heat stress (HS) condition, TW%, DMC% and FTW presented high heritability (>0.70), the others presented high heritability, with values between 0.51 and 0.97 for Pn-1DAS and Fv/Fm-35DAS, respectively. Considering the genotype x environment interaction, the heritability values were from low to high magnitude, with values between 0.0018 and 0.6583, for C_b and NPQ-15DAS respectively (Table11). The observed accuracy values ranged from 0.08 to 0.94, being considered low to high accuracy, according to Resende and Duarte (2007). Around 80% of the measured traits showed the highest accuracy values, while the variable with the lowest value were for the C_a, C_b and C_c measured after 1DAS. (Table11).

Table 10: Estimates of the components of variance, heritability, accuracy and relative reduction for the variables: net photosynthesis rate (Pn), stomatal conductance rate (Gs), transpiration rate (Tr), effective photochemical quantum yield of PSII (YII), non-photochemical quenching (NPQ) , maximum quantum efficiency of the PSII (Fv/Fm), chlorophyll A (Ca), chlorophyll B (Cb), carotenoid contents (Cc), on the first day after stress (1DAS), at 15DAS, 35DAS and total water % (TW%), fresh tuber weight (FTW), dry matter content % (DMC) at the end of the plant life cycle of potato (*Solanum* sect. *Petota*, Solanaceae) accessions from Embrapa Potato Genebank.

Traits	DAS	Treatment	σ_g^2	σ_e^2	σ_{int}^2	H_g^2	A_g	Mean
Pn	1DAS	CT	11.8397	5.6170		0.6782	0.899	11.1954
		HS	5.0303	4.7706		0.5132	0.8236	11.1971
		G x T	2.6715	5.1936	5.7642	0.196	0.6244	11.1963
	15DAS	CT	14.1775	3.5941		0.7978	0.9421	8.6733
		HS	18.4996	2.547		0.879	0.9673	6.7173
		G x T	9.7006	3.0705	6.6380	0.4998	0.8388	7.6953
	35DAS	CT	1.3502	4.7446		0.2215	0.6023	7.9485
		HS	5.6544	2.4684		0.6961	0.906	10.548
		G x T	1.8735	3.6064	1.6289	0.2635	0.7224	9.2483
Gs	1DAS	CT	0.0048	0.0020		0.7100	0.9113	0.1322
		HS	0.0133	0.0033		0.8017	0.9434	0.1519
		G x T	0.0062	0.0026	0.0030	0.5245	0.8594	0.1399
	15DAS	CT	0.0149	0.0032		0.8245	0.9507	0.1636
		HS	0.0083	0.0006		0.9279	0.9811	0.0863
		G x T	0.0046	0.0019	0.007	0.3399	0.7319	0.1249
	35DAS	CT	0.0038	0.0031		0.5527	0.8438	0.1475
		HS	0.0132	0.0085		0.6087	0.8699	0.2851
		G x T	0.0052	0.0058	0.0033	0.3664	0.7935	0.2163
Tr	1DAS	CT	0.6468	0.2378		0.7312	0.9191	1.9225
		HS	1.1787	0.3799		0.7563	0.928	2.0429
		G x T	0.7553	0.3102	0.1920	0.6007	0.9006	1.9523
	15DAS	CT	1.5177	0.2064		0.8803	0.9676	2.0620
		HS	3.0199	0.2051		0.9364	0.9834	2.0239
		G x T	1.147	0.2058	1.1218	0.4635	0.8074	2.0429
	35DAS	CT	0.3026	0.2853		0.5147	0.8244	1.8463
		HS	1.2161	0.9552		0.5601	0.8474	4.907
		G x T	0.4685	0.6203	0.2908	0.3396	0.7805	3.3766
YII	1DAS	CT	0.0019	0.0004		0.8209	0.9495	0.1132
		HS	0.0014	0.0003		0.8342	0.9537	0.0961
		G x T	0.0005	0.0003	0.0011	0.2742	0.6796	0.1046
	15DAS	CT	0.0026	0.0002		0.9176	0.9783	0.1200
		HS	0.002	0.0002		0.8914	0.9709	0.1163
		G x T	0.0017	0.0002	0.0006	0.6545	0.9023	0.1181
	35DAS	CT	0.0032	0.0002		0.9404	0.9845	0.1151
		HS	0.0039	0.0003		0.9319	0.9822	0.1849
		G x T	0.0008	0.0002	0.0028	0.2024	0.5835	0.1508
NPQ	1DAS	CT	0.0081	0.0012		0.8708	0.9648	0.6662
		HS	0.016	0.0012		0.9303	0.9818	0.6736
		G x T	0.008	0.0012	0.0040	0.6065	0.8815	0.6699
	15DAS	CT	0.0124	0.0006		0.9508	0.9873	0.6469
		HS	0.0104	0.0006		0.9473	0.9864	0.6686
		G x T	40.9578	5.8193	15.441	0.6583	0.9039	18.1

	35DAS	CT	0.0109	0.0012		0.8989	0.9730	0.6922
		HS	0.0144	0.0005		0.9645	0.9909	0.579
		G x T	18.8345	5.9911	65.704	0.2080	0.5904	23.0233
Fv/Fm	1DAS	CT	0.0046	0.0001		0.9718	0.9928	0.7441
		HS	0.003	0.0002		0.9257	0.9805	0.7033
		G x T	0.0012	0.0002	0.0027	0.2886	0.6755	0.7237
	15DAS	CT	0.0032	0.0001		0.9740	0.9934	0.7424
		HS	0.0002	0.0000		0.9516	0.9875	0.7623
		G x T	0.0000	0.0000	0.0016	0.0258	0.2257	0.7524
	35DAS	CT	0.0006	0.0000		0.9558	0.9886	0.7549
		HS	0.0007	0.0000		0.9745	0.9935	0.7496
		G x T	0.0001	0.0000	0.0006	0.1666	0.5384	0.7522
Chl.a	1DAS	CT	0.0060	0.2919		0.0203	0.1994	1.4642
		HS	0.0484	0.1746		0.2171	0.5973	1.4096
		G x T	0.0009	0.2348	0.0251	0.0033	0.1087	1.4369
	15DAS	CT	0.0741	0.3007		0.1976	0.5745	1.9312
		HS	0.0255	0.2076		0.1093	0.4438	2.0851
		G x T	0.0316	0.2537	0.0187	0.1038	0.55	2.0082
	35DAS	CT	0.0108	0.1737		0.0585	0.3324	1.9403
		HS	0.0673	0.3263		0.1709	0.5403	2.3057
		G x T	0.0307	0.2489	0.0094	0.1061	0.5606	2.123
Chl.b	1DAS	CT	0.0048	0.6222		0.0077	0.1235	0.7948
		HS	0.0059	0.0468		0.1124	0.4495	0.645
		G x T	0.0006	0.3366	0.0028	0.0018	0.084	0.7199
	15DAS	CT	0.0058	0.0651		0.0824	0.3902	0.8836
		HS	0.0084	0.0463		0.1542	0.5169	0.8924
		G x T	0.0077	0.0543	0.0008	0.1228	0.5962	0.888
	35DAS	CT	0.0003	0.0424		0.0072	0.1193	0.7945
		HS	0.0094	0.0705		0.1179	0.4592	0.9685
		G x T	0.0071	0.0538	0.0004	0.1156	0.5844	0.8815
Carot	1DAS	CT	0.0032	0.0032		0.5023	0.8177	0.2885
		HS	0.0019	0.0054		0.2656	0.6479	0.2957
		G x T	0.0001	0.0043	0.0024	0.0215	0.2468	0.2922
	15DAS	CT	0.0031	0.0081		0.2740	0.6559	0.3634
		HS	0.0007	0.0088		0.0736	0.3703	0.4015
		G x T	0.0001	0.0084	0.0018	0.0064	0.1463	0.3824
	35DAS	CT	0.0024	0.0046		0.3379	0.7107	0.3578
		HS	0.0003	0.0122		0.0243	0.2178	0.4055
		G x T	0.0007	0.0085	0.0006	0.0734	0.479	0.3817
TW%		CT		0.1401	25.66		0.0054	0.1039	83.8062
		HS		17.7622	8.3208		0.681	0.9001	81.4386
		G x T		5.3808	19.2607	1.2897	0.2075	0.7045	82.6224
FTW		CT		3074.86	1570.46		0.6619	0.8925	89.9340
		HS		1236.242	422.1817		0.7454	0.9242	53.3919
		G x T		2246.403	1015.87	69.543	0.6742	0.9411	69.1325
DMC%		CT		9.9689	1.7345		0.8518	0.9591	25.1632
		HS		13.1347	5.9853		0.687	0.9025	24.0753
		G x T		7.2742	3.8776	3.8823	0.4838	0.8427	24.6578

4.4.3. Genotypic values

The genotypes were ranked, for each evaluated variable, in each control and stress condition, according to its predicted genotypic value. Genotypes with the highest predicted genotypic value were ranked first and with low value ranked last as shown in **figure 19-29** in both control and stress conditions for all traits measured after 1DAS, 15DAS and 35DAS.

4.4.3.1. Net photosynthesis rate (Pn):

For gas exchange traits, such as Pn average, genotypic values were reduced with the pasage of time. High values were observed after 1DAS and then they reduced after 15 days, reaching the lowest values after 35 days in control condtions. However, under HS conditions, average genotypic values for Pn showed the same trend but increase was observed after 35DAS (**Figure 19**). BGB077, under CT conditions, showed the highest genotypic values for Pn-1DAS and 35DAS. BGB011 showed highest after 15DAS in both CT and HS conditions. While the lowest were observed for BGB100, BGB110, BGB094 after 1DAS, 15DAS and 35DAS, respectively. In HS treatment, BGB099 has the highest predicted values after 1DAS and 35DAS, and the lowest ones were obsereved for the BGB110, BGB095, BGB094 for 1DAS, 15DAS and 35DAS.

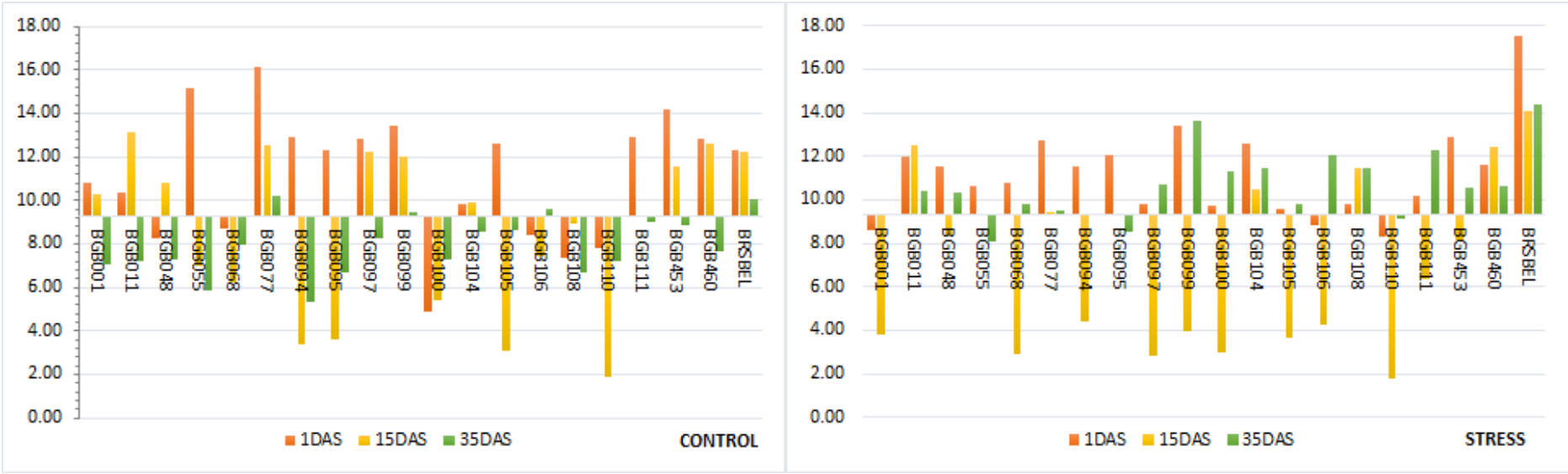

Figure 19: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the Pn variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

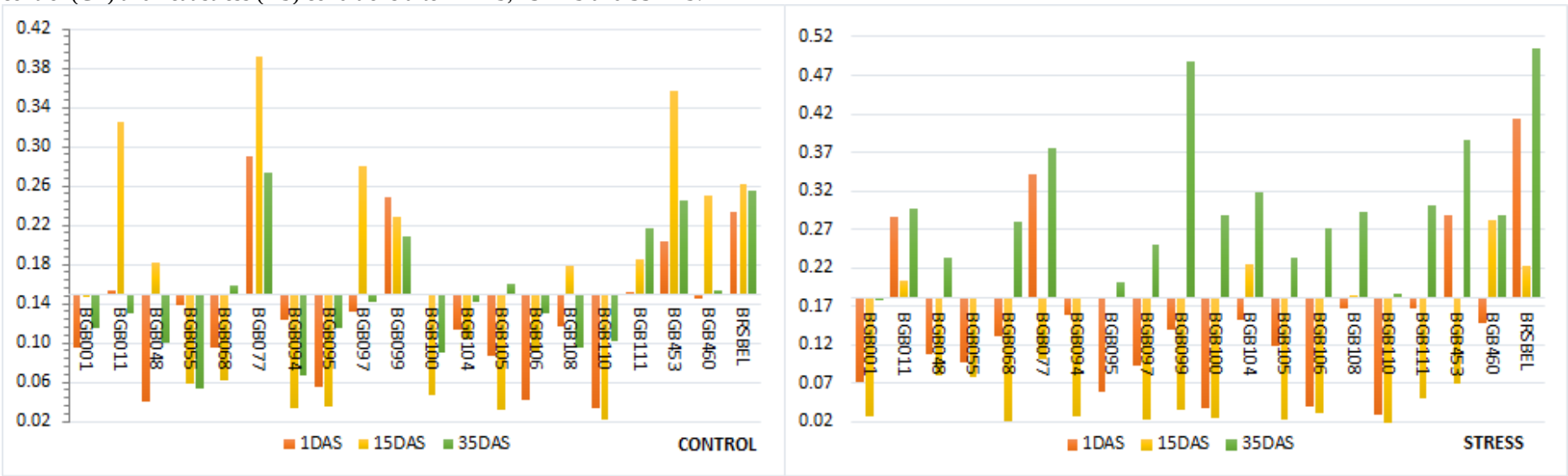

Figure 20: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the Gs variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

4.4.3.2. Stomatal Conductance Gs:

In CT, after 1DAS stomatal coductance was lowest while after 15DAS and 35DAS Gs activity was increased. While, in HS conditions, after 1DAS stomatal conductnce was high which becomes lower after 15DAS and again it raises on average after 35DAS. BGB077 maintened the highest predicted genotypic values under CT conditions but under HS it is only able to maintain high genotypic values until 1DAS and BGB460 ranked first after 15DAS while BGB099 ranked first after 35DAS (**Fugure 20**). The lowest genotypic values were observed for BGB100, BGB110, BGB055 under CT and BGB110, BGB095, BGB055 under HS condition after 1DAS, 15DAS, 35DAS, respectively.

4.4.3.3. Transpiration Rates (Tr):

According to predicted genotypic values for transpiration rates measured after 1DAS, 15DAS and 35DAS showed the same trend as shown for the Gs under both CT and HS conditions. Also, the same genotype BGB077 showed the highest genotypic values for Tr and lowest for the BGB100, BGB110, BGB055 under control conditions. However, under HS conditions, BGB001 ranked first and BGB460 ranked last after 1DAS, 15DAS and 35DAS (**Figure 21**).

4.4.3.4. Effective photochemical quenching of PSII (YII):

Average of the predicted genotypes values showed a linear trend thorughout the life cycle of potato genotypes under CT conditions. In HS conditions, average of genotypic values for YII showed a increasing trend when measured after 1DAS and follwed by 15DAS, 35DAS. BGB460 ranked first after 1DAS and 15DAS and it was ranked last when measured after 35DAS under CT. BGB011 after 1DAS and BGB111 ranked first after 15DAS, 35DAS. Lowest predicted genotypic values are showed by BGB105, BGB100 and BGB048 respectively after 1DAS, 15DAS and 35DAS (**Figure 22**).

4.4.3.5. Non-photochemical quenching (NPQ):

The average for the predicted genotypic values in both conditions CT and HS almost showed a linear trend, except when measured after 35DAS-HS when a slight decrease in the average of NPQ is showed. BGB001 was first after 1DAS in both CT and HS, and BGB110 was first after 15DAS, 35DAS in CT, and BGB099 and BGB100 were first in HS after 15DAS, 35DAS, respectively (**Figure 24**).

Figure 21: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the Tr variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

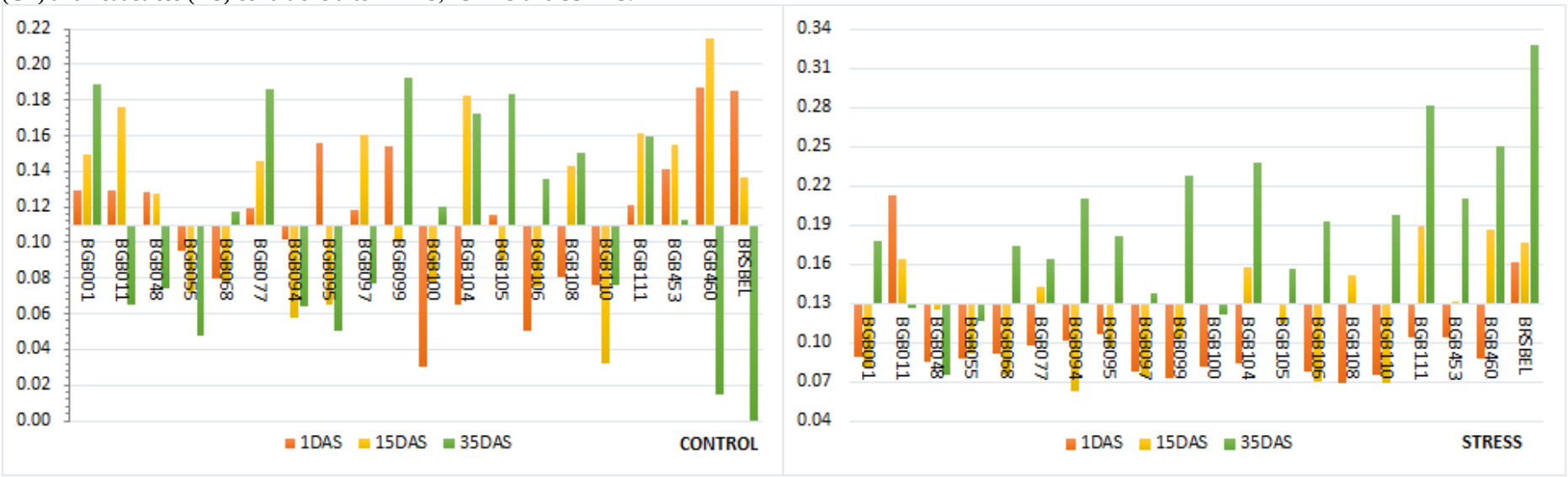

Figure 22: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the YII variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

4.4.3.6. Maximum photochemical yield of PSII (Fv/Fm):

Under CT condition there was no diferrence in average of the predicted genotypic values for Fv/Fm after 1DAS, 15DAS and 35DAS. Under HS conditions, genotypic values were lower after 1DAS, and highest after 15DAS and slight lower again after 35DAS. BGB453, BGB055 and BGB108 ranked first for predicted genotypic values for Fv/Fm in HS treatment after 1DAS, 15DAS and 35DAS, respectively (**Figure 23**).

4.4.3.7. Chlorophyll Contents (C_a, C_b, C_c):

Average of predicted genotypic values for C_a (Figure 22), C_b (Figure 23), and C_c (Figure 24) traits showed a increase with prolongation of stress period or life cycle of potato plants under both growth conditions. In case of individual genotypes BGB099 and BGB100 were always ranked first according to the predicted genotypic values under heat stress conditions (**Figure 25, 26, 27**).

4.4.3.8. Total water content % (TW%):

Predicted genotypic values for TW% showed a reduction when measured under HS condtions. The highest genotyic value was obserevd for BGB001 under both growing environments (CT & HS). The lowest genotypic values were obsered for the BGB110 in CT and BGB097 in HS conditions (**Figure 28**).

4.4.3.9. Tuber traits (FTW, DMC%):

For the both tuber traits, there was reduction in the average of the predicted genotypic values observed under HS as compare to CT conditions (**Figure 29**). Genotypic values observed for FTW was shown highest by BGB097 under CT and HS. BGB460 lowest genotypic values under CT and BGB077 showed lowest and negative predicted genotypic values under HS environment. In case of DMC%, BGB100 and BGB011 ranked first with highest genotypic values and BGB068, BGB110 observed lowest and ranked last according to genotypic values for DMC under CT and HS conditions, respectively.

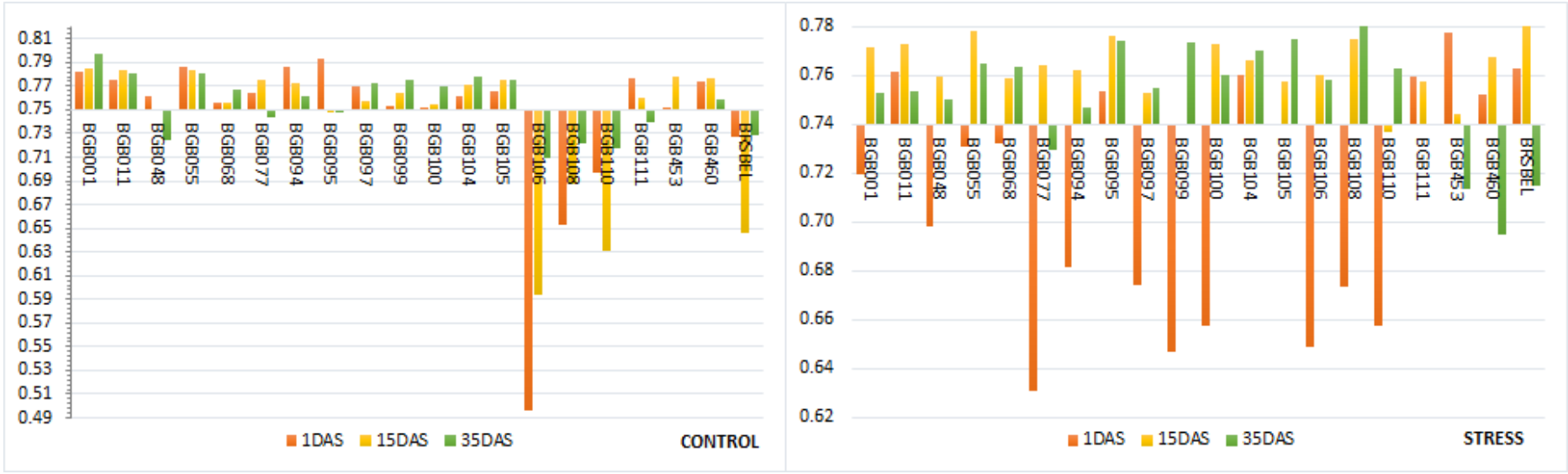

Figure 23: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the Fv/Fm variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

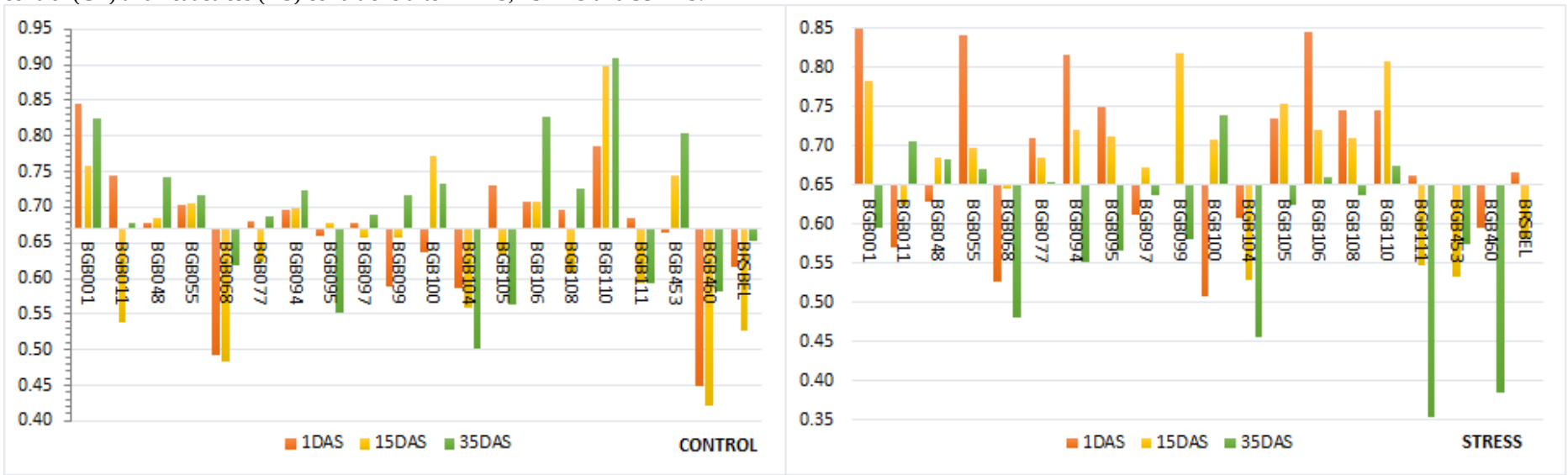

Figure 24: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the NPQ variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

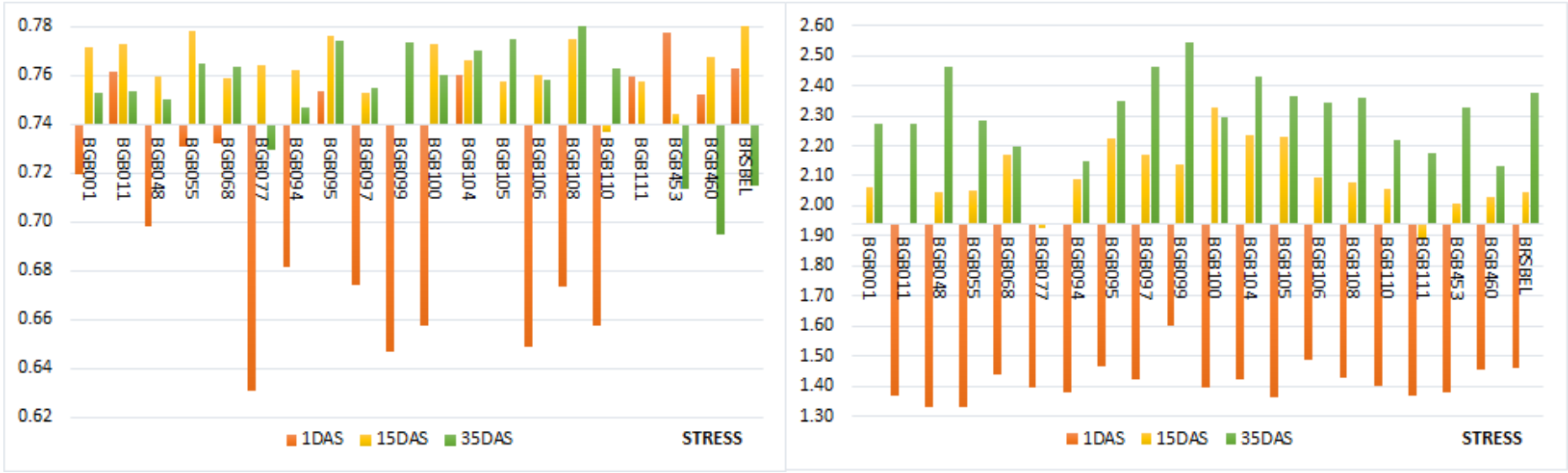

Figure 25: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the Ca variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

Figure 26: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the Cb variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

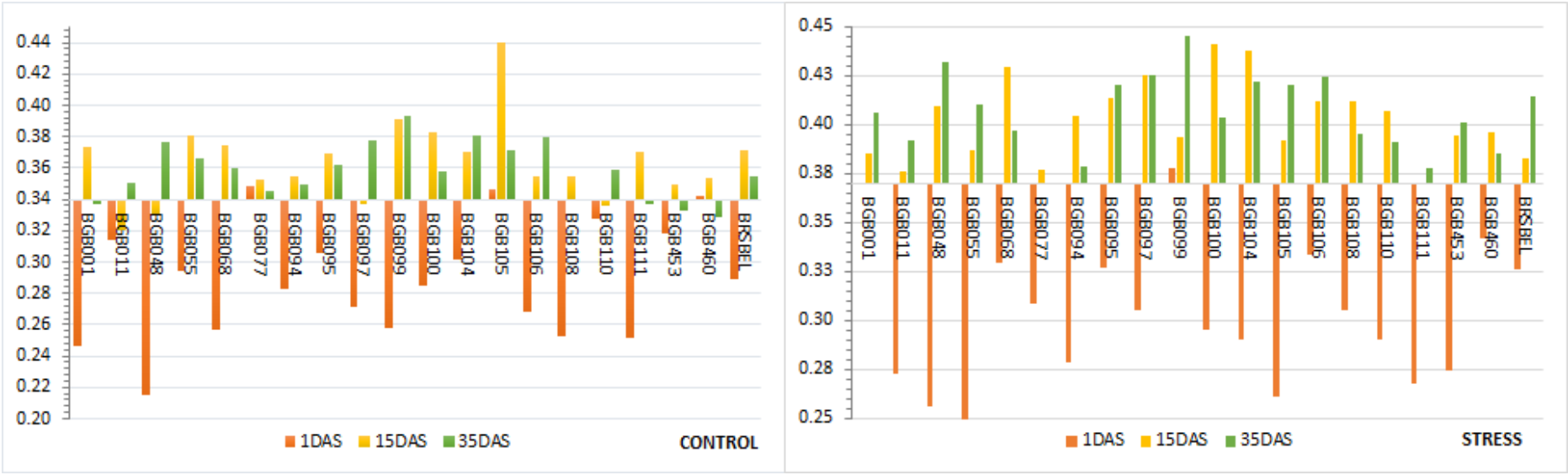

Figure 27: Predicted genotypic values in 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the Cc variable under control (CT) and heat stress (HS) conditions after 1DAS, 15DAS and 35DAS.

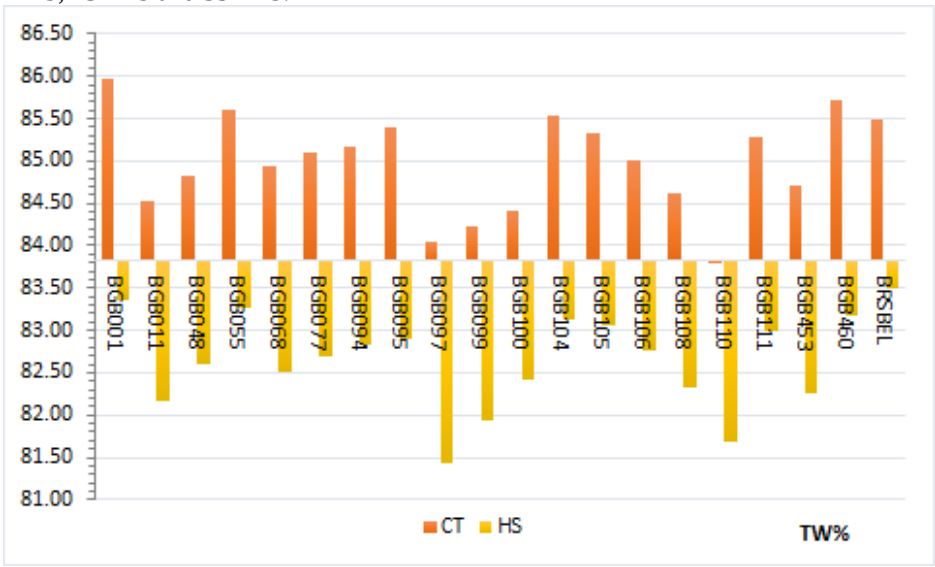

Figure 28: Predicted genotypic values in 20 potato (Solanum sect. Petota, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the TW% variable under control (CT) and heat stress (HS) conditions.

Figure 29: Predicted genotypic values in 20 potato (Solanum sect. Petota, Solanaceae) germplasm accessions from Embrapa Potato Genebank, for the FTW and DMC% variable under control (CT) and heat stress (HS) conditions.

4.4.4. Principal component analysis (PCA):

4.4.4.1. Morpho-physiological variables – Control

By analyzing the principal components of the morpho-physiological data, in the control condition, the first two components explained 43.10% of the variation, with 28.38% in the first component and 14.72% in the second component (**Figure 30**).

The variables that most contributed to the separation of genotypes in the first and second component were Tr, Gs, Pn, YII, Fv/Fm measured after 1DAS, Tr, Gs, Pn, YII, Fv/Fm, C_b measured after 15DAS, Gs, Tr measured after 35DAS.

Cluster analysis under control condition divided the used genotypes into three clusters. Cluster 1 contains BGB048, BGB106, BGB108 and BGB110; cluster 2 encompasses BGB001, BGB055, BGB068, BGB094, BGB095, BGB100 and BGB104; and cluster 3 include genotypes BRSBEL, BGB011, BGB077, BGB097, BGB099, BGB111, BGB453 and BGB460. From cluster score calculation, cluster 1 showed the highest value to allow the assumption that genotypes belonging to the cluster 1 showed better performance under tested enivronmental conditions.

4.4.4.2. Morpho-physiological variables – Heat Stress

By principal component analysis of the morpho-physiological data in the heat stress condition, the first two components explained 42.21% of the variation, with 27.98% in the first component and 14.23% in the second component (**Figure 32**). The variables that most contributed to the separation of genotypes in the first and second components were Pn, Gs, Tr, YII, C_a, C_b, C_c under 1DAS, Pn, Gs, Tr, YII under 15DAS, Gs, Tr, NPQ for (35DAS) and FTW.

In cluster analysis, Inertia gain by ward's method suggested that under stress conditions, accessed genotypes are grouped into nine clusters. Cluster 2 contain the most of the genotypes. However, cluster 4 contain one genotype BGB099, that has the highest cluster score. So, it can be characterized as the most tolerant genotype under heat stress condition (**Table 13**).

4.4.5. Correlation between variables:

Under both control (**Figure 34**) and heat stress (**Figure 35**) conditions, gas exchange variables (Pn, Gs, Tr) and YII were significantly high correlated with each other when measured after 1DAS, 15DAS and 35DAS, while chlorophyll A, B and carotenoids showed high significant correlation. However, chlorophyll A and B has high negative correlation with gasous exchange traits. Tuber traits such as FTW has medium significant negative association with TW% and DMC% has positiive significant correlation with FTW under control conditions but no significant

correlation found under heat stress. Under stress conditions, NPQ showed medium to high signifcant negative correlation with Pn, Gs, Tr, YII for 15DAS and 35DAS.

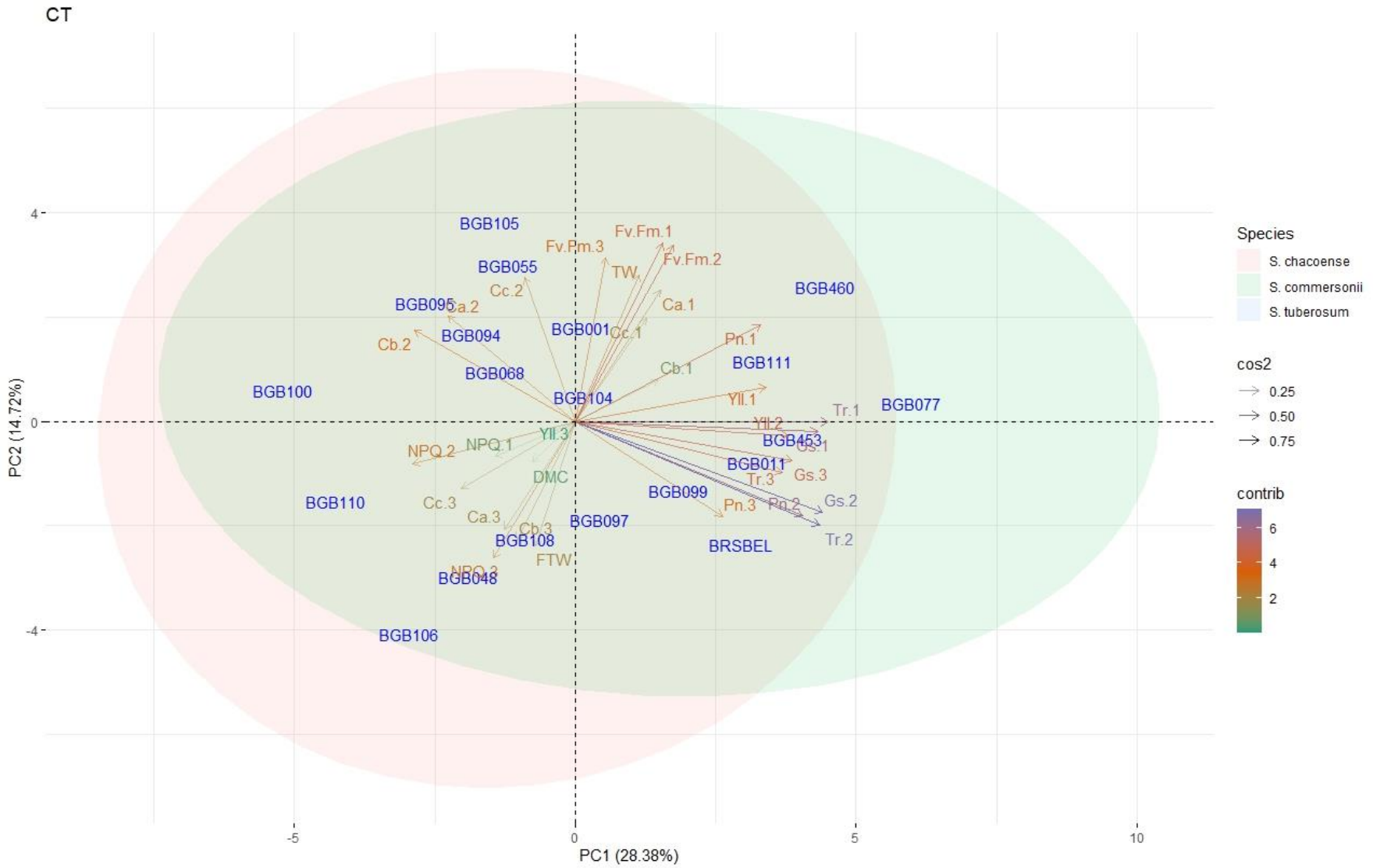

Figure 30: Dispersion of 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank by principal component analysis in the control (CT) condition for the morpho-physiological variables: net photosynthesis rate (Pn), stomatal conductance rate (Gs), transpiration rate (Tr), effective photochemical quantum yield of PSII (YII), non-photochemical quenching (NPQ) , maximum quantum efficiency of the PSII (Fv/Fm), chlorophyll A (Ca), chlorophyll B (Cb), carotinoid contents (Cc), on the first day after stress (1DAS), at 15DAS, 35DAS and total water % (TW%), fresh tuber weight (FTW), dry matter content % (DMC) at the end of the plant life cycle.

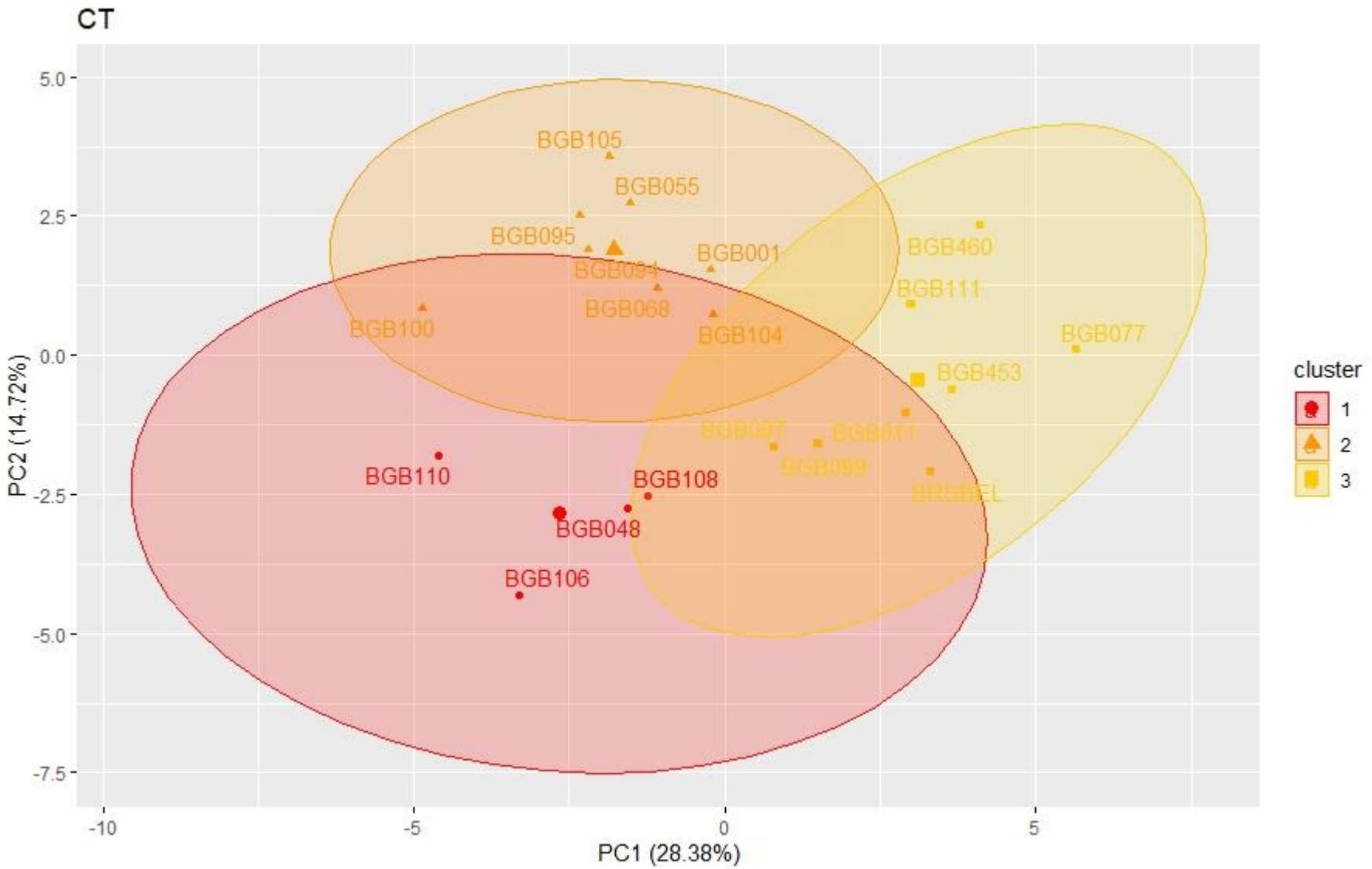

Figure 31: Clustering of 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank under control temperature (CT) condition for the morpho-physiological variables responses measured after 1DAS, 15DAS, 35DAS and at the end of the life plant life cycle.

Table 11: Eigenvector values of 9 morpho-physiological variables evaluated in the control measured after 1DAS, 15ADS and 35DAS. PC1= first principal component; PC2= second principal component. Pn: net photosynthesis rate, Gs: stomatal conductance rate, Tr: transpiration rate, YII: effective photochemical quantum yield of PSII, NPQ: non photochemical quencing, Fv/Fm: maximum quantum efficiency of the PSII, Ca: chlorophyll A, Cb: chlorophyll B, Cc: carotenoid contents, TW%: total water %, FTW: fresh tuber weight, DMC%: dry matter content %.

Treatment		**Control Treatment** (CT)		**Heat Stress (HS)**	
DAS	**Variables**	**PC1**	**PC2**	**PC1**	**PC2**
1DAS	Pn	0.6585	0.3691	0.7717	0.1865
	Gs	0.8619	-0.0397	0.8683	-0.1257
	Tr	0.8986	-0.0006	0.8699	-0.1535
	YII	0.6772	0.1305	0.5966	-0.2572
	NPQ	-0.2841	-0.1332	-0.3466	-0.2127
	Fv/Fm	0.3108	0.6812	0.3394	-0.1303
	Chl.*a*	0.3026	0.5032	0.3144	0.6921
	Chl.*b*	0.2960	0.1585	0.3027	0.6066
	Carot	0.2527	0.3952	0.3466	0.7410
15DAS	Pn	0.8070	-0.3596	0.8150	-0.3120
	Gs	0.8796	-0.3482	0.7871	-0.2124
	Tr	0.8694	-0.3972	0.8051	-0.2566
	YII	0.7594	-0.0542	0.8291	-0.1606
	NPQ	-0.5765	-0.1608	-0.5774	0.1498
	Fv/Fm	0.3505	0.6757	-0.0083	-0.5466
	Chl.*a*	-0.4535	0.4029	-0.3983	0.3502
	Chl.*b*	-0.5720	0.3491	-0.4147	0.3482
	Carot	-0.1798	0.5492	-0.2679	0.0608
35DAS	Pn	0.5237	-0.3641	0.4003	0.3564
	Gs	0.7718	-0.1500	0.6841	0.4965
	Tr	0.7366	-0.1969	0.5878	0.5137
	YII	-0.0027	0.0052	0.5501	0.2343
	NPQ	-0.2895	-0.5227	-0.6570	-0.1310
	Fv/Fm	0.1086	0.6243	-0.2736	-0.0031
	Chl.*a*	-0.2528	-0.4130	-0.2381	0.3348
	Chl.*b*	-0.2075	-0.4554	-0.2455	0.3009
	Carot	-0.4043	-0.2583	-0.2704	0.3184
TW %		0.2270	0.5599	0.2229	-0.4893
FTW		-0.1411	-0.4764	0.0889	0.6523
DMC%		-0.1533	-0.1562	-0.0031	0.4353

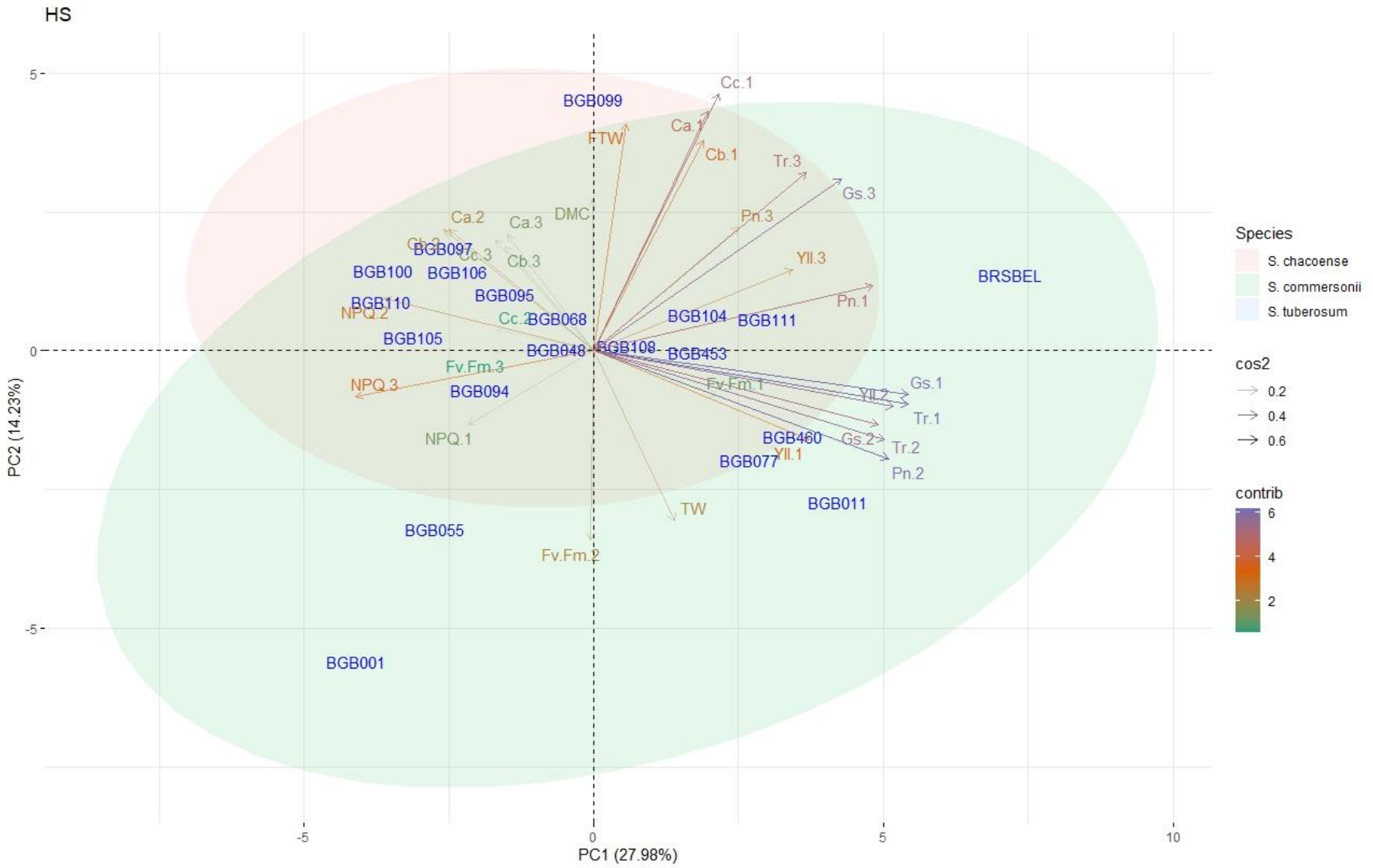

Figure 32: Dispersion of 20 potato (Solanum sect. Petota, Solanaceae) germplasm accessions from Embrapa Potato Genebank by principal component analysis in the heat stress (HS) condition for the morpho-physiological variables: net photosynthesis rate (Pn), stomatal conductance rate (Gs), transpiration rate (Tr), effective photochemical quantum yield of PSII (YII), non-photochemical quenching (NPQ) , maximum quantum efficiency of the PSII (Fv/Fm), chlorophyll A (Ca), chlorophyll B (Cb), carotenoid contents (Cc), on the first day after stress 1DAS, 15DAS, 35DAS and total water % (TW%), fresh tuber weight (FTW), dry matter content % (DMC) at the end of the plant life cycle.

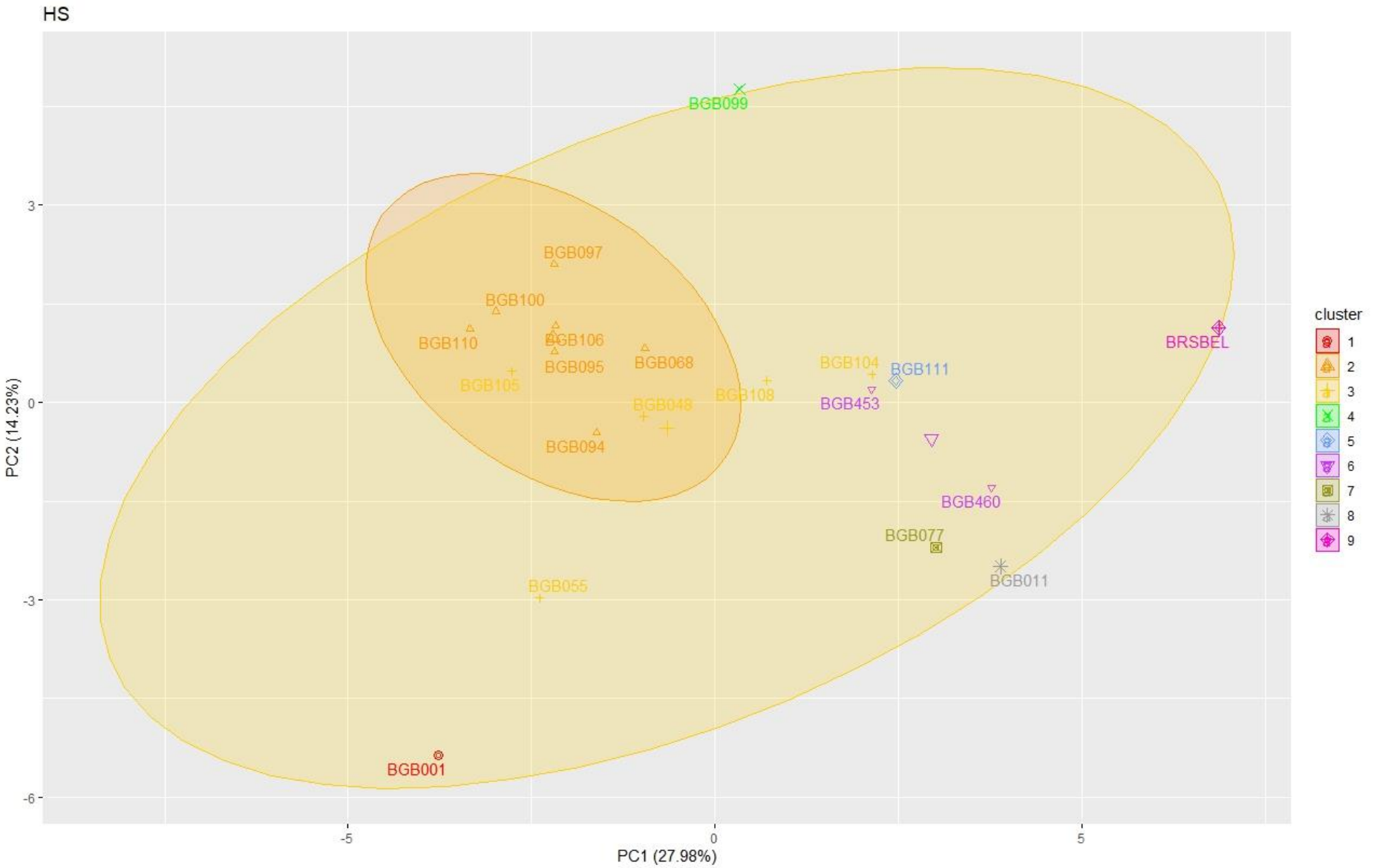

Figure 33: Clustering of 20 potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank under heat stress (HS) condition for the morpho-physiological variables responses measured after 1DAS, 15DAS, 35DAS and at the end of the life plant life cycle.

Table 12: Descriptive statistics of physiological traits of wild potato (*Solanum* sect. *Petota*, Solanaceae) genotypes for different clusters under control (CT) and heat stress (HS) conditions.

DAS	Variable	Control (CT)			Heat Stress (HS)								
		Score1	Score2	Score3	Score1	Score2	Score3	Score4	Score5	Score6	Score7	Score8	Score9
1DAS	Pn	6.95	9.52	11.45	7.84	9.26	9.88	12.23	9.31	11.18	11.62	10.91	16.02
	Gs	0.05	0.08	0.17	0.07	0.07	0.12	0.13	0.16	0.21	0.32	0.27	0.39
	Tr	0.87	1.25	2.33	1.35	1.11	1.71	1.97	2.77	2.48	3.32	2.86	3.56
	YII	0.07	0.08	0.11	0.09	0.08	0.06	0.07	0.10	0.09	0.09	0.20	0.15
	NPQ	0.53	0.50	0.47	0.80	0.64	0.67	0.61	0.62	0.28	0.67	0.54	0.63
	Fv.Fm	0.54	0.64	0.64	0.66	0.63	0.53	0.59	0.70	0.70	0.58	0.70	0.70
	C_a	1.26	1.31	1.34	0.00	1.40	1.35	1.57	1.34	1.39	1.37	1.34	1.43
	C_b	0.72	0.72	0.72	0.00	0.63	0.63	0.64	0.64	0.63	0.63	0.63	0.63
	C_c	0.22	0.24	0.24	0.00	0.29	0.26	0.36	0.25	0.29	0.29	0.26	0.31
15DAS	Pn	6.93	5.81	11.37	3.45	2.50	7.91	3.59	5.77	9.42	8.63	11.42	12.87
	Gs	0.12	0.06	0.28	0.03	0.02	0.11	0.03	0.05	0.17	0.10	0.20	0.22
	Tr	1.65	0.99	3.18	0.75	0.59	2.66	0.94	1.39	3.61	2.74	4.43	4.52
	YII	0.08	0.08	0.13	0.07	0.06	0.11	0.09	0.17	0.14	0.12	0.14	0.15
	NPQ	0.66	0.6	0.54	0.66	0.60	0.57	0.69	0.46	0.22	0.58	0.53	0.49
	Fv.Fm	0.62	0.72	0.71	0.41	0.40	0.40	0.00	0.40	0.40	0.40	0.41	0.41
	C_a	1.74	1.90	1.75	1.96	2.06	2.03	2.04	1.8	1.92	1.84	0.00	1.95
	C_b	0.71	0.76	0.70	0.85	0.89	0.86	0.88	0.79	0.83	0.79	0.00	0.84
	C_c	0.31	0.35	0.32	0.38	0.41	0.40	0.38	0.00	0.38	0.37	0.37	0.37
35DAS	Pn	7.18	6.69	8.26	6.60	6.26	7.26	9.71	8.75	7.53	6.75	7.43	10.24
	Gs	0.10	0.11	0.19	0.17	0.23	0.21	0.48	0.29	0.33	0.36	0.29	0.49
	Tr	1.42	1.41	2.18	2.89	3.67	3.21	5.42	4.31	4.52	4.48	4.18	5.49
	YII	0.09	0.1	0.09	0.15	0.14	0.12	0.19	0.23	0.19	0.13	0.1	0.27
	NPQ	0.72	0.59	0.61	0.56	0.58	0.58	0.54	0.33	0.45	0.61	0.66	0.00
	Fv.Fm	0.61	0.65	0.64	0.74	0.75	0.76	0.76	0.00	0.69	0.72	0.74	0.70
	C_a	1.87	1.83	1.83	2.26	2.27	2.37	2.53	2.16	2.22	0.00	2.26	2.36
	C_b	0.69	0.67	0.67	0.95	0.95	0.99	1.03	0.91	0.94	0.00	0.98	0.97
	C_c	0.29	0.28	0.28	0.40	0.40	0.41	0.44	0.38	0.39	0.00	0.39	0.41
	TW	72.82	73.45	73.09	0.00	44.6	27.50	82.09	67.50	0.00	-2.27	-0.49	87.59
	FTW	81.46	45.41	69.40	0.00	18.18	15.12	16.28	19.42	0.00	21.11	22.39	13.93
	DMC	19.93	15.96	17.49	71.39	70.54	70.99	70.18	71.08	70.85	70.82	70.37	71.52
	Total Score	211.21	172.76	211.18	105.48	170.21	159.78	216.46	202.08	122.45	137.17	144.51	239.61

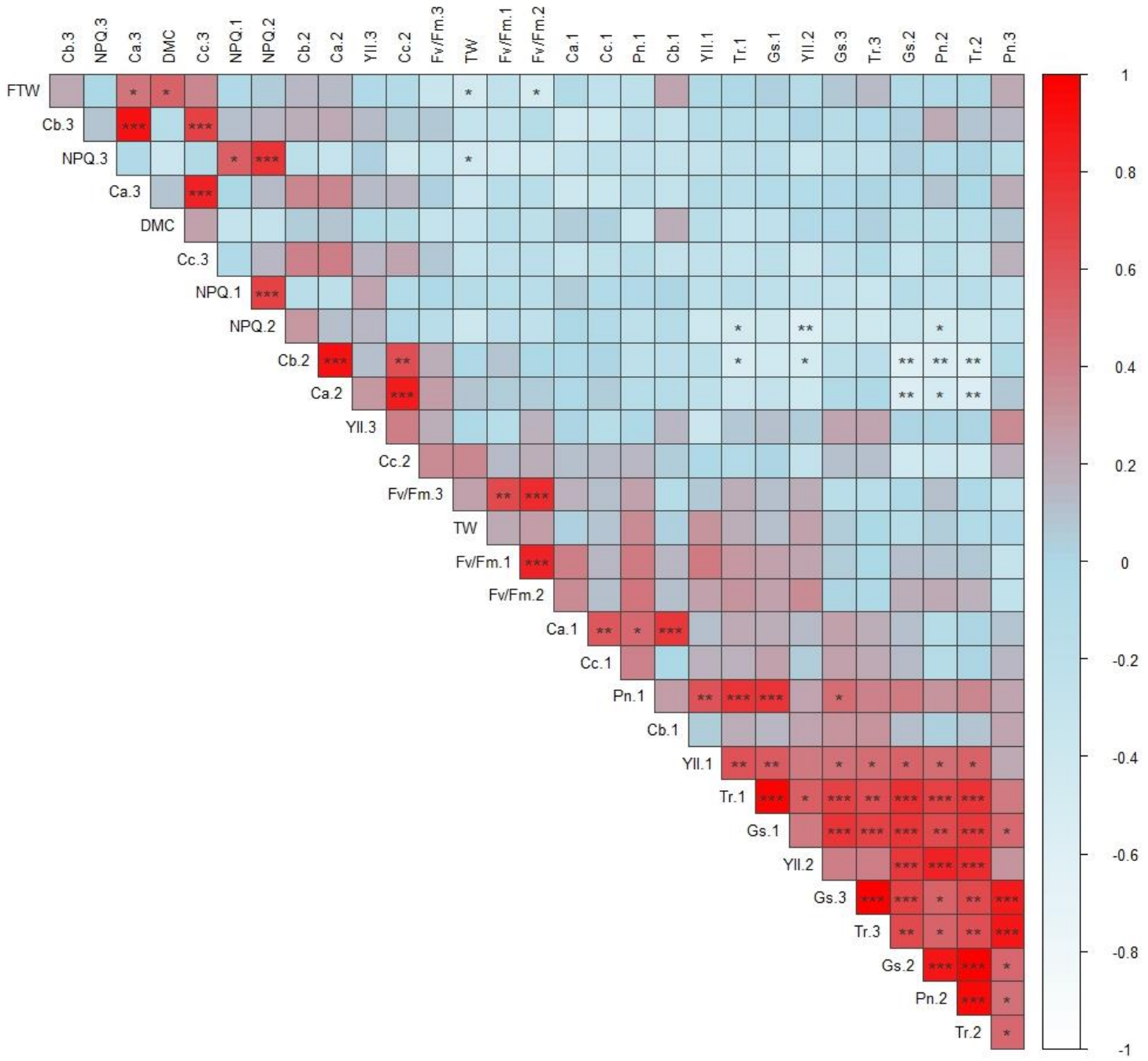

Figure 34: Correlation between variables net photosynthesis rate (Pn), stomatal conductance rate (Gs), transpiration rate (Tr), effective photochemical quantum yield of PSII (YII), non-photochemical quenching (NPQ) , maximum quantum efficiency of the PSII (Fv/Fm), chlorophyll A (Ca), chlorophyll B (Cb), carotenoid contents (Cc), on the first day after stress 1DAS, 15DAS, 35DAS and total water % (TW%), fresh tuber weight (FTW), dry matter content % (DMC) at the end of the plant life cycle potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank under CT conditions.

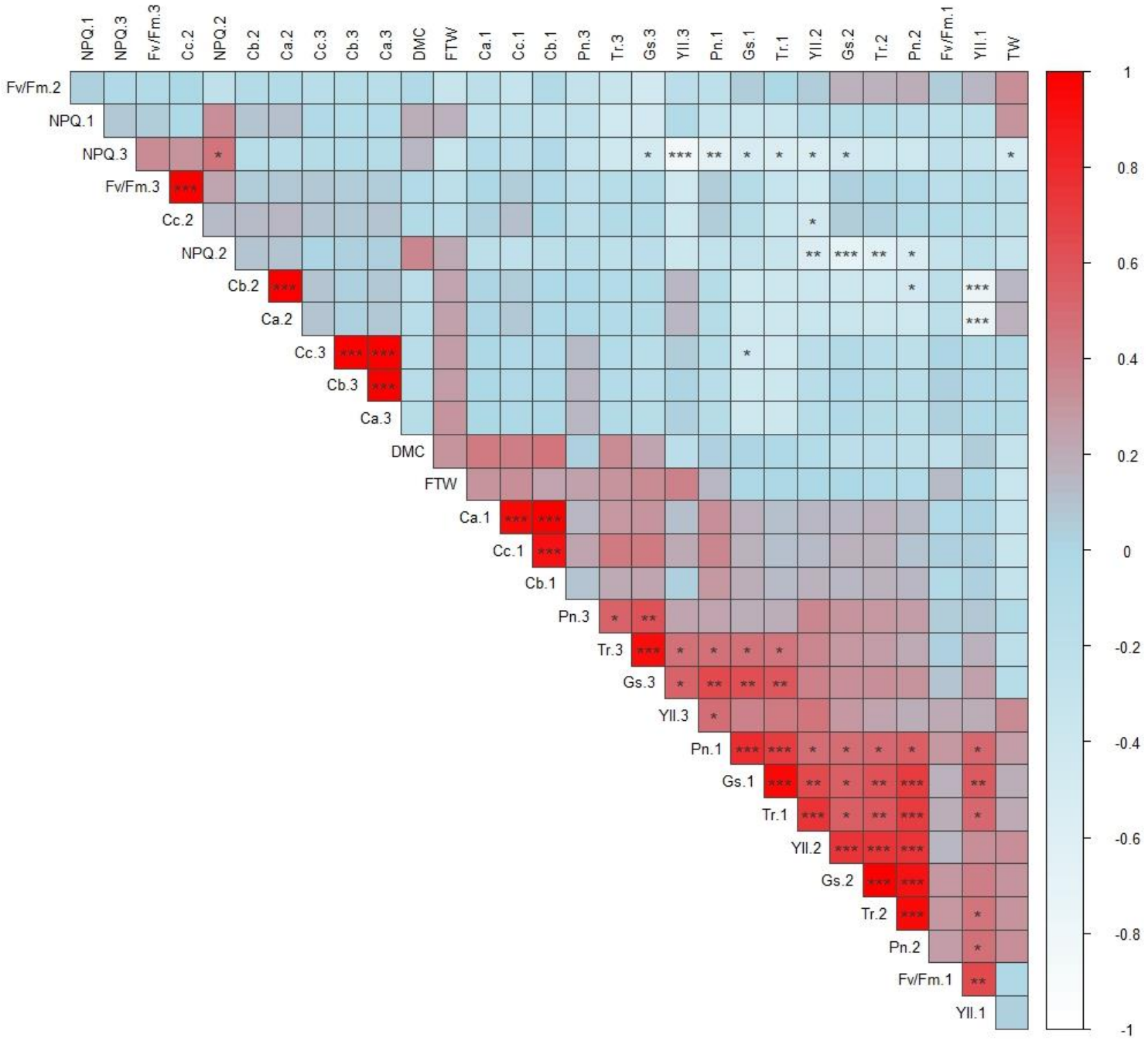

Figure 35: Correlation between variables net photosynthesis rate (Pn), stomatal conductance rate (Gs), transpiration rate (Tr), effective photochemical quantum yield of PSII (YII), non-photochemical quenching (NPQ) , maximum quantum efficiency of the PSII (Fv/Fm), chlorophyll A (Ca), chlorophyll B (Cb), carotenoid contents (Cc), on the first day after stress 1DAS, 15DAS, 35DAS and total water % (TW%), fresh tuber weight (FTW), dry matter content % (DMC) at the end of the plant life cycle potato (*Solanum* sect. *Petota*, Solanaceae) germplasm accessions from Embrapa Potato Genebank under HS conditions.

4.5. Discussion

The results obtained confirm the effect of increasing temperature on the gas exchange, chlorophyll fluorescence and chlorophyll contents in potato genotypes and show that there are differences between the evaluated accessions. Photosynthetic efficiency is among the physiological mechanisms related to heat tolerance in potatoes (WOLF et al., 1990). In tomatoes, changes in photosynthetic activity clearly demonstrate susceptibility to high temperature (CAMEJO et al., 2005). In species such as wheat, good yield under heat stress is associated with maintenance of photosynthesis rate, chlorophyll content and stomatal conductance at elevated temperatures (YANG et al., 2002). In potatoes, the optimal temperature for photosynthesis is around 24°C, above which there is a reduction in the efficiency of the PSII, an increase in maintenance of respiration and a reduction of the leaf area (AIEN et al., 2011; PRANGE et al., 1990). According to Burton (1981), an increase of 5°C in the optimal temperature causes a decrease of about 25% in the photosynthetic rate. Other studies with higher temperatures reductions in the photosynthetic rate ranging from 37% to 45% in heat-sensitive genotypes, when evaluated after heat treatment at 40°C compared to the rate at 20°C, were observed (HAMMES; DE JAGER, 1990; REYNOLDS et al., 1990). However, Hancock et al. (2013) evaluating potato plants exposed to slightly higher temperatures, 30 and 20°C (day/night), observed an increase in the net rate of carbon assimilation, which agrees with results previously observed by Dwelle et al. (1981), who suggest that moderately high growing temperatures, up to approximately 30°C, have a positive impact on photosynthesis of cultivars described as heat tolerant. The elevated temperature of 34°C used in this experiment did not significantly affects the photosynthesis rate of the wild genotypes in relation to the temperature of the control condition, however these genotypes increased the stomatal conductance and the transpiration rate. The increase in stomatal conductance observed, mainly in the genotypes belonging to *S. commersonii*, was also observed by other authors in several potato genotypes (DEMIREL et al., 2017; DWELLE et al., 1981; HANCOCK et al., 2013). According to Demirel et al. (2017), the increase in stomatal conductance under hot conditions may be related to the transpiration cooling mechanism under high temperature conditions, favored by the required water availability in which the plants grow.

Higher temperature conditions are possibly related to damage to the PSII reaction centers, as confirmed by the response to the maximum quantum efficiency of the PSII. Changes in fluorescence emission from chlorophyll a in photosynthetic organisms are the result of changes

in photosynthetic activity, mainly changes in mesophyll capacity, which depend on Rubisco activity and photosynthetic electron transport capacity to regenerate Rubisco (BAKER; ROSENQVIST, 2004; FELLER et al., 1998). The wild genotypes, when cultivated in higher temperature conditions, had not shown a significant reduction in the effective quantum yield of the PSII Y(II), when compared to the control condition, indicating that the temperature did not influence the photochemical efficiency of the given genotypes evaluated here. Therefore, the dissipation of light energy captured by chlorophyll directed to photosynthesis is higher, with lower dissipation in the form of heat (CHAVES, 2015). In this study the effect of heat stress temperature on the photosynthetic activity of the wild genotypes was not reflected in reductions in both shoot and tuber yield, as well as in dry matter content. However, in general, the reduction of the photosynthetically active area due to the reduced efficiency of PSII in plants under heat stress directly affects the final tuber production (FAGUNDES et al., 2010; PRANGE et al., 1990). Hence, It is possible to say that reduction in the tuber yield or other agronomic traits in wild genotypes under heat stress involved some tuber mechanisms because addition to the decline in photosynthesis, the increase in stomatal conductance and transpiration under high temperature conditions result in a decrease in tuber production (DEMIREL et al., 2017) as was shown in this study.

The appearance of the tuber is of paramount importance, while in industry, characteristics that confer frying quality, such as high dry matter content, low reducing sugar content and absence of disturbances physiological factors are important (SILVA et al., 2014). The dry matter content varies according to the genotype and is influenced by the growing conditions. Under conditions of high temperature there is stimulation of shoot development, reducing the partition of photoassimilates to the tubers, producing smaller tubers with low dry matter content (MENEZES et al., 1999; MENEZES et al., 2001).

The maximum effective quantum yield of PSII (Fv/Fm) is a parameter of chlorophyll fluorescence, which has been used to monitor the physiological status of plants after a period of high temperature treatment (RYKACZEWSKA, 2015). For unstressed leaves, values above 0.8 are considered optimal (MURCHIE; LAWSON, 2013). Among evaluated wild potato genotypes, *Solanum chacoense* genotypes showed values near to optimal values to estimate whether leaves were stressed or not. In the following evaluations, the average values for the Gs and Fv/Fm became higher in the stress condition in relation to the control condition, thus demonstrating the effect of the genotypic resistance of wild genotypes. In high temperature environments, the increase in Tr usually occurs as a cooling mechanism, as the loss of water helps to remove heat from the leaves (TAIZ; ZEIGER, 2017). In all the evaluations carried out,

the Tr was superior in the heat stress condition in relation to the control condition, demonstrating that all genotypes are losing more water, possibly allowing reducing their temperature.

Chlorophyll a is the main pigment involved in light collection in PSI; in PSII, both chlorophyll a and b are important. The increase in the chlorophyll a/b ratio in plants exposed to stress indicates possible changes in the PSI/PSII ratio, due to mechanisms of adaptation of chloroplasts to stress (Takabayashi et al. 2005). Increases in the PSI ratio increase cyclic electron transport, which is involved in the dissipation of excess energy, thus preventing further damage to the PSII (Li et al. 2006). In addition, the light curve made it possible to confirm the strong limitation of photosynthetic reactions that respond to the increase in light intensity, a fact corroborated by the reduction in the electron transport rate. The excess of absorbed energy was dissipated through regulated energy dissipation mechanisms, that is, through the xanthophyll cycle (Müller et al. 2001), since there was inhibition of non-regulated mechanisms. It has been shown that the mechanism of energy dissipation through the xanthophyll cycle can be effective in defending against different types of stress (Baroli and Niyoji 2000). Moreover, since chlorophyll content is an indicator of early senescence, it is positively correlated with crop yield (Araus et al., 1997; Rharrabti et al., 2001). In this study, wild potato genotypes showed higher production of chlorophyll a, b and carotenoids content under heat stress conditions as compared to control conditions, which also explain higher rate of Pn and other stress indicators evaluated in this experiment. Moreover, carotenoids protect plants from photooxidative stress. In recent years, metabolic engineering efforts have also been undertaken with the aim to improve plant resistance to abiotic stress through overproduction of carotenoids (Giuliano et al., 2008).

4.6. Conclusion

In general, higher temperatures decreased total tuber mass and leaf area while increasing the gas exchange variable in the studied wild potato genotypes.

Under stress, however, genotypes with higher tuber production had increased transpiration rate, stomatal conductance, and chlorophyll fluorescence indicators.

According to cluster analysis, under HS conditions, BGB099 obtained the highest cluster score by using true genotypic values. It could be indicated as the most tolerant genotype under elevated temperature conditions.

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5. Concluding Remarks

Potato is one of the most important crops used all over the world as a staple food or a snack, which increases its importance to make it available as needed. Climate change is one of the most significant challenges for this crop. Abiotic stresses, specifically heat and drought stress. All other bacterial, viral, and pre- or post-harvest losses in potato crops are always the result of these stresses. To address this issue in this era, the first step in potato breeding is to investigate genetic diversity and identify resistant genes or germplasm. In this regard, wild potato genotypes are the best available option to select and invest in to search for novel traits to deal with abiotic stresses and broaden potato genetic basis.

Latin America is home to wild potato relatives. One of the most important keepers of this important source of diversity is the Embrapa potato Genebank. As a result, the completion of this work makes significant contributions to the development of potato germplasm that is more adaptable to the climate change scenario. Heat stress responses in plants are the result of a complex set of actions and interactions between various mechanisms. Key factors for understanding how potato genotypes respond to heat stress were identified in this study.
Wild potatoes are a large genetic reservoir for potato breeding, providing countless genes for novel traits and resistances to abiotic and biotic stresses not found in commercial cultivars. Screening for desirable traits in germplasm conserved at genebanks is well documented, and some traits have been successfully introgressed into modern potato cultivars. We provide a comprehensive review of current and potential uses of wild potatoes for breeding, organized into three major topics: commercial traits, abiotic stress resistance (frost, drought, heat, salinity), and biotic stress resistance (bacterial, fungal, and viral diseases, and insects and nematodes pests). The challenges to overcome to fully realize the potential of potato wild relatives for potato breeding are briefly summarized. Finally, the promising future of wild potatoes in breeding is discussed, as no other crop has as many wild relatives in its gene pool as potatoes. The increased accessibility and development of new biotechnological techniques have facilitated and expanded the potential of using wild potatoes for breeding cultivated potatoes, and the use of wild relatives is more promising than ever.

In the second study published in potato research, we attempted to establish the fact that many wild potatoes do not bear tubers of quality to be ready consumed and, worse, they have undesirable tuber characteristics that should not be dragged to the breeding lineages. Anyway, using mixed model methodologies, it is still possible to identify the best performing genotypes

in terms of tuber yield under unfavorable conditions. Because wild potatoes require very specific conditions for tuberization, high temperature treatments were used in the experiment to observe the response of physiological parameters such as gas exchange and chlorophyll fluorescence, among which various stress indicators were accessed to identify the resistant genotypes under heat stress.

Photosynthetic activity is an important factor to study under heat stress in *Solanaceae* crops such as potato. Overall, in the study of evaluating potato wild relatives (*Solanum* sect. Petota) germplasm diversity for photosynthetic traits response under heat stress found significant variation in HS tolerance among potato genotypes, and several relatively heat stress tolerant and sensitive genotypes were identified based on physiological traits. Combining physiological traits measured by chlorophyll fluorescence analysis with Li-Cor (IRGA) traits would be ideal for genotype screening. This type of non-invasive screening procedure allows recognizing genotypes with overall higher stress tolerance, *i.e*., at the physiological level; such genotypes can then be used as parental genotypes in breeding programs to develop terminal potato heat tolerant genotypes.

In the further exploration of wild potato genotypes for their genetic parameters and responses associated with high temperature effects evaluation in potato wild relatives, the higher temperature mainly caused reductions in the variable total tuber mass, and an increase in the gas exchange variable. In the stress condition, genotypes with higher tuber yield generally showed higher transpiration rate, stomatal conductance, and chlorophyll fluorescence indicators. According to cluster analysis, under HS conditions, BGB099 obtained the highest cluster score by using true genotypic values. It could be indicated as the most tolerant genotype under elevated temperature conditions.

This project is a foundation for future studies in potato genetic improvement to identify resistant genotypes that perform well under abiotic stresses and introduce them into breeding programs for the genetic improvement of *Solanum tuberosum* in face of anticipated climate change.

Further exploratory analyses are possible to be carried out, based on the data already presented in this thesis, providing guidance to the choice of the best wild potatoes accessions that has potential traits that may allow develop a climate-proof potato cultivar resilient to heat and drought abiotic stresses.