Screening of Diploid Wild Potatoes Pollen Traits under Heat Stress

Rodrigo Nicolao *, Ikram Bashir, Caroline Marques Castro, Gustavo Heiden

Posted Date: 3 November 2023

doi: 10.20944/preprints202311.0176.v1

Keywords: Crop wild relatives; diploid breeding; pre-breeding; Solanaceae; variability

Article

Screening of Diploid Wild Potatoes Pollen Traits under Heat Stress

Rodrigo Nicolao [1], Ikram Bashir [1], Caroline M. Castro [2] and Gustavo Heiden [2]

[1] Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (UFPel). Pelotas-RS 96010-900, Brazil. ikrambashir215@gmail.com

[2] Embrapa Clima Temperado. BR-392, km 78, Pelotas, RS 96010-971, Brazil; caroline.heiden@embrapa.br (C.M.C.); gustavo.heiden@embrapa.br (G.H.)

* Correspondence: rodnicolao@gmail.com

Abstract: Screening of pollen traits in diploid wild potatoes (*Solanum* sect. *Petota*, Solanaceae) is desirable to develop heat-tolerant potato (*S. tuberosum*) cultivars. To accomplish this goal requires exploring potato genetic resources that are conserved in genebanks. The goal of this study was to assess pollen viability and 2n pollen production of the diploid potato wild relatives under heat stress condition. We assessed pollen viability and size of nine potato accessions conserved at the Embrapa Potato Gene Bank, including *S. chacoense* (BRA 00167447-2, BRA 00167017-3, BRA 00167023-1, BRA 00167028-0), *S. commersonii* (BRA00167007-4, BRA00167420-9, BRA00183760-8), and *S. malmeanum* (BRA 00183755-8), along with a control accession from the cultivated species *S. tuberosum* (BRA 00167251-8). The plant accessions were cultivated in different growth chambers, simulating both control temperature (ranging from 14 to 27°C) and supraoptimal temperature conditions (ranging from 24 to 34°C). At heat stress, the accessions BRA 00167251-8 did not bloom, and BRA 00167023-1 did not produce pollen. The remaining accessions did not exhibit a significant reduction in pollen viability as the temperature increased. Pollen viability at the control temperature had the lowest value in BRA-00167420-9 (*S. commersonii*) with 68.5% and the highest in BRA 00183755-8 (*S. malmeanum*) with 100%. At the supraoptimal temperature the lowest value was in BRA 00167420-9 (*S. commersonii*) with 54.5% and the highest in BRA 00183755-8 (*S. malmeanum*). The average of pollen size was 20 µm in all wild potato genotypes, and the increase of temperature did not lead to 2n pollen production. Estimated Genotypic Coefficient of Variation (GCV) was lower than Phenotypic Coefficient of Variation (PCV) for pollen viability. The observed heritability values ranged from 58.82% in BRA00167007-4 to 91.32% in BRA 00183755-8. Our results highlight the genetic variability available in wild potato germplasm concerning pollen viability under heat stress. Furthermore, these first insights offer valuable guidance for ongoing and future endeavors in diploid potato breeding.

Keywords: Crop wild relatives; diploid breeding; pre-breeding; Solanaceae; variability

1. Introduction

As F1-hybrid potato breeding continues to progress, the assessment of pollen viability becomes an essential trait in germplasm screening[1]. In regions with tropical climates characterized by high temperatures, the identification of genotypes with robust blooming and appropriate pollen viability under supraoptimal temperatures can be advantageous for the cultivation of True Potato Seeds (TPS) propagated cultivars[2]. In the context of global climate change, exploring the phenotypic plasticity of crops in response to rising temperatures is crucial for anticipating potential heat-related effects[3]. Considering future climate change scenarios projecting a 1 to 3°C increase in global average temperatures during the twenty-first century[4,5], there is a potential for substantial reductions in agricultural production and impacts on natural populations of crop wild relatives in their natural distribution areas, including potatoes[6,7].

Globally, potato (*Solanum tuberosum* L., Solanaceae) ranks as the third most important crop, trailing only rice and wheat[8,9]. Despite the genetic diversity of the potato crop is relatively limited[10], potatoes and their wild relatives encompass a phylogenetic branch comprising 107 wild species, four domesticated species, and their natural and artificial hybrids[11]. Natural populations

 doi:10.20944/preprints202311.0176.v1

of potato wild species have evolved in extreme environmental conditions such as in arid regions, high temperatures, intense sunlight, water scarcity, and high altitudes, given their extensive geographical distribution from the southwestern United States to Mexico, Peru, and the coastal regions of Chile, including Brazil[12,13]. The genetic variability is crucial for maintaining and improving the genetic foundation of agricultural crops[14], and can be found in the crop wild relatives, which are considered natural sources of genes with agronomic, industrial, and nutritional significance[15–17].

The domesticated and wild potatoes are members of *Solanum* sect. *Petota*, which can reproduce sexually, involving botanical seeds, also referred to as true-potato-seeds (TPS), and vegetatively, via stolons and tubers[18,19]. Particularly, the ability to generate diploid inbred lines through TPS[9,20,21] stands in contrast to the prevailing practice of predominantly tetraploid commercial potato crops (2n=4x=48), which are propagated through vegetative manner by tubers[20]. Diploid potato cultivars propagated through TPS help prevent the transmission of viruses to the next generation and facilitates the storage and conservation of propagative material[21,22]. Even more, along with the potential utility of an F1-hybrid population for genetic mapping studies[23–25]. This contrast has piqued significant interest among breeders, farmers and for the seed industry towards the development of F1-hybrid potato cultivars[22,26,27]. This heightened interest is further fueled by the accessibility of modern plant breeding techniques and genetic tools[1,28,29].

The effect of heat stress can lead to the unreduced (2n) gametes formation in many crop species such as tomato (*Solanum lycopersicon* L.)[30] and wheat (*Triticum turgidum* L.)[31], causes pollen unviability[32], and can cause disruptions in the stability of the natural populations[33].

By investigating the impact of temperature on pollen viability and 2n pollen production in diploid potato wild relatives, our study aims to contribute to the identification of genotypes that can thrive in high-temperature environments. This research is crucial for the development of potato cultivars that can withstand the challenges posed by global climate change.

2. Materials and Methods

2.1. Plant Material

This study used nine wild potato accessions from the species *S. chacoense* (BRA 00167447-2, BRA 00167017-3, BRA 00167023-1, BRA 00167028-0), *S. commersonii* (BRA00167007-4, BRA00167420-9, BRA00183760-8), and *S. malmeanum* (BRA 00183755-8), as well as a control accession from of the commercial cultivar of *S. tuberosum* named BRSIPR-BEL (BRA 00167251-8) from the Embrapa Potato Gene Bank (Table 1).

Tubers of uniform size were planted in a 5L plastic container with TurfaFértil® organo-mineral substrate and supplemental fertilizer according to the crop instructions. The plants were transported to the growth chambers after 15 days of growth and exposed to two temperature gradients: control temperature (Figure 1A), with thermal amplitude ranging from 14 to 27°C; and supraoptimal temperature (Figure 1B), with amplitude ranging from 24 to 34°C (Figure 1B). The photoperiod was 12 hours (7:00 to 19:00) with a light intensity of about 400 mol m^{-2} s^{-1}. The plants stayed under these two distinct treatments until harvest.

A double factorial experimental scheme 9 × 2 (genotype vs. temperature), was set up in randomized blocks with two replications each consisting of one plant.

Table 1. Accessions of wild potato species (*Solanum* sect. *Petota*, Solanaceae) conserved at Embrapa Potato Gene Bank evaluated in this study. Heading information is followed by local code, genesys code, Species, origin (City, state, country), latitude (Lat) and longitude (Lon) in UTM.

Local code	**Genesys code**	Species	Origin	Lat	Lon
BGB447	BRA00183755-8	*S. malmeanum*	Porto Lucena, RS, Brazil	-27.8561	-55.0164
BGB100	BRA 00167017-3	*S. chacoense*	Catamarca, Argentina	-41	-71.5

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 November 2023 doi:10.20944/preprints202311.0176.v1

BGB110	BRA 00167028-0	*S. chacoense*	Unknown	-	-
BGB106	BRA 00167023-1	*S. chacoense*	Unknown	-	-
BGB095	BRA 00167447-2	*S. chacoense*	Cordoba, Argentina	-31.13333	-64.48333
BRSIPR-BEL	BRA00167251-8	*S. tuberosum*	Brazil	-	-
BGB001	BRA00167007-4	*S. commersonii*	Ijuí, RS, Brazil	-28.388	-53.915
BGB453	BRA00183760-8	*S. commersonii*	Herval, RS, Brazil	-32.0236	-53.3956
BGB068	BRA00167420-9	*S. commersonii*	São Gabriel, RS, Brazil	-30.336	-54.32

2.2. Pollen Viability Assessment

Five flowers in the anthesis stage (when the floral bud opens) from each accession were collected on the same day for anther extraction and pollen processing[34]. All the accessions from the two treatments opened the flowers in the same week, and pollen was collected seven days after the anthesis. Pollen from each plant was collected and preserved in 1.5 mL tubes in a freezer (-4°C) until analysis. To determine pollen viability, the percentage of staining pollen (PSP) was accessed in 400 grain samples dyed with red aceto-carmine glycerol[35]. The examination of samples, as described in the technique provided by the protocol of CIP (International Potato Center)[36] and demonstrated in Figure 1C, was conducted using an optical microscope with a 200x magnification.

The formula for calculating viability (percent) is according to:

$$Viability\ (\%) = \frac{Number\ of\ stained\ pollen\ grains}{Total\ number\ of\ pollen\ grains} \times 100$$

Based on the viability range data obtained, four distinct categories were defined to classify pollen viability[36]. The percentage of pollen viability was considered sterile (0%), low (> 0% and ≤ 50%), moderate (< 50% and ≤ 80%), or high (< 80% and ≤ 100%).

2.3. Pollen Size

To distinguish 2n pollen, pollen grains were stained with a droplet of 2% (w/v) aceto-carmine solution on a microscope slide. Enlarged pollen grains were classified as 2n pollen if their diameter exceeded 1.2 times the average diameter of the pollen grains in the control group[36]. For analysis of pollen size, pollen from each accession subjected to both temperature treatments were collected, following the protocol described by CIP[36].

2.4. Statistical Analysis

The standard error and significant differences ($p \leq 0.05$) for temperature, genotype, and genotype interaction were determined using analysis of variance (ANOVA), for pollen viability and pollen size. Following that, the Tukey mean comparison test was used, and the analyses were carried out using the function *fat2.dbc* of the 'ExpDes.pt' package[37] in the RStudio environment[38].

2.4.1. Pollen Viability-Based Heat Susceptibility Index (HSIpv)

The pollen viability-based heat susceptibility index (*HSIpv*) was calculated in Microsoft Excel 2023 using percent pollen viability values of heat stress and normal conditions following the formula below[39,40]:

$$HSIpv = 1 - \left(\frac{Xstress}{Xnormal}\right)/1 - \left(\frac{\bar{x}stress}{\bar{x}normal}\right)$$

Building upon, *HSIpv* genotypes were grouped into three classes: tolerant (*HSIpv* <0.5), moderately tolerant (*HSIpv* 0.5-0.99), and susceptible (*HSIpv* >1.0).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 November 2023 doi:10.20944/preprints202311.0176.v1

2.4.2. Genetic Parameters

The basic genetic parameters – PCV%, GCV%, h² (broad sense), genetic gain (GG) and (genetic advance) GA were calculated. The phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) were calculated using the formulas: $PCV\ (\%) = \frac{\sqrt{Vp}}{mean} \times 100$ and $GCV\ (\%) = \frac{\sqrt{Vg}}{mean} \times 100$, respectively.

Where PCV is phenotypic coefficient of variance, VP is phenotypic variance, GCV is genotypic coefficient of variance, and Vg is genotypic variance. To categorize the GCV and PCV values, the classification proposed by Burton[41] was employed. GCV and PCV values falling within the range of 0 to ≤10% were classified as low, values ranging from 10 to ≤20% were categorized as moderate, and values exceeding 20% were considered high.

2.4.3. Heritability

Estimated as the ratio of total genotypic variance to the phenotypic variance[42]:

$$H^2 = \frac{Vg}{Vp} \times 100$$

where $H2$ = % broad sense heritability. The heritability percentage was categorized as low (0–30%), moderate (30–60%), and high ≥60% as given[43]:

$$GG\% = \frac{GA}{\sum} \times 100$$

The genetic gain was calculated by the ratio of genetic advance ($GA = (k \times \sqrt{V_p} \times H^2)$, k represents the constant value 2.06 at 5% selection intensity and overall mean (**∑**) of the population used.

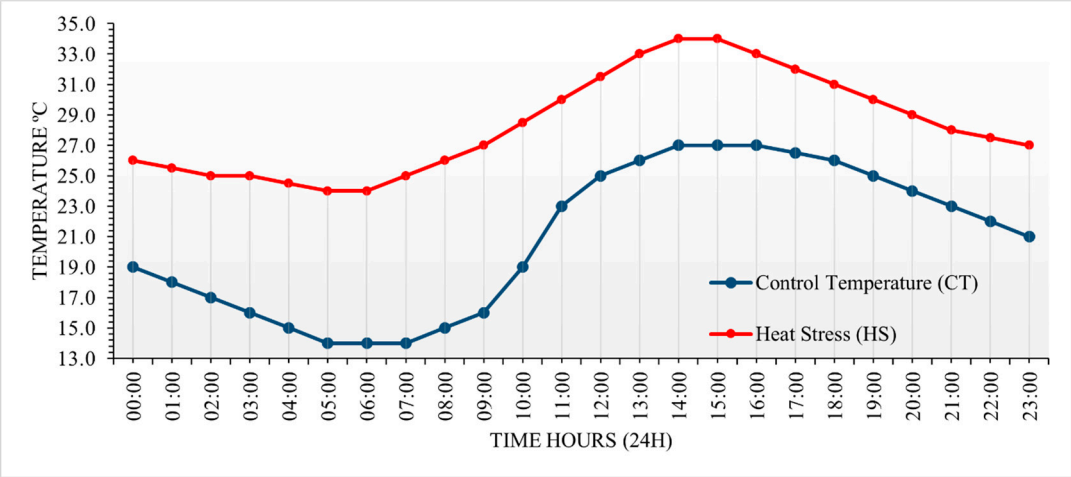

Figure 1. Temperature profiles of the control condition (represented by the blue line) and heat stress condition (represented by the red line).

 doi:10.20944/preprints202311.0176.v1

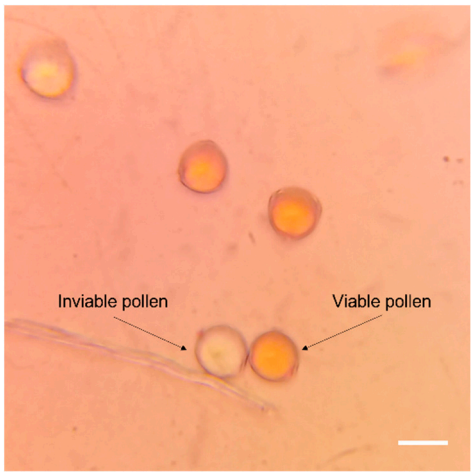

Figure 2. Pollen viability was assessed under an optical microscope using red acetic carmine staining (scale bar = 20 µm). Arrows indicate nonviable (uncolored) and inviable (colored) pollen grains.

3. Results and Discussion

3.1. Analysis of Variance

The results of the ANOVA indicated that both main effects, block, genotype and temperature, and the G×E interaction (Table 2). A non-significative reduction in pollen viability was observed with increasing temperature in genotypes BRA00167007-4, BRA00167420-9, BRA 00167447-2, BRA 00167017-3, BRA 00167028-0, BRA 00183755-8 and BRA00183760-8. Conversely, BRA 00167023-1 failed to produce pollen, and BRA 00167251-8 did not bloom under elevated temperatures; hence, these two genotypes were not included in this analysis.

Table 2. Analysis of Variance (ANOVA) for pollen viability of wild potatoes (*Solanum* sect. *Petota*, Solanaceae) from Embrapa Potato Genebank cultivated at both control (CT) and heat stress conditions (HS).

	Df	Sum Sq	Mean Sq	F-value	Pr (>F)
Block	1	82.3	5	2.450	0.141
Genotype	6	4278.7	4	21.228	0.000*
Temperature	1	869.1	6	25.872	0.000*
Genotype×Temperature	6	340.9	3	1.532	0.200
Residue	13	436.7	2		
Total	27	6007.7	1		
CV (%)	7.13				

Head column is followed by Block, Genotype, Temperature, the interaction Genotype × Temperature, Residue, Total, and Coefficient of Variation (CV %). Head of row is followed by Df: Degree of Freedom, Sum Sq: Sum of Squares, Mean Sq: Mean squares, F-value: F distribution, Pr(>F): *p*-value at <0.05%. (*) significant to 5% probability.

3.2. Pollen Viability-Based Heat Susceptibility Index (HSIpv)

In the Table 3 are presented the genetic parameters estimated for pollen viability. GCV (15.76%) was lower than PCV (17.84%). Following Johnston et al.[44] genetic advance (% average) is categorized as low (<10%), moderate (10 – 20%) and high (>20%). In our study the genetic advance for pollen viability was high (23.30%) (Table 3).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 November 2023 doi:10.20944/preprints202311.0176.v1

Table 3. Genetic Parameter Estimates for Pollen Viability of wild potatoes (*Solanum* sect. *Petota*, Solanaceae) from Embrapa Potato Genebank under Control (CT) and Heat Stress (HS) conditions.

Components		Pollen viability
V_P	phenotypic variance	210.29
V_G	genotypic variance	164.10
Ve	residual variance	35.66
$V_{G\times T}$	genotype × treatment interaction variance	10.54
H^2_B	broad sense heritability (%)	78
A_G	accuracy in genotypic selection	0.96
PCV	phenotypic coefficient of variation	17.84
GCV	genotypic coefficient of variation	15.76
GG	genetic gain %	28.68
GA	Genetic advance	23.30
∑	General average	81.29

Pollen viability of the potato wild genotypes from the two experimental conditions are presented in Table 4. As the G×E interaction was non-significative, the Tukey test was only applied for each treatment. At the CT, genotypes BRA 00183755-8, BRA 00167017-3, BRA 00167028-0, BRA 00167447-2, and BRA00167007-4 exhibited the highest pollen viability values. Conversely, genotypes BRA00167420-9 and BRA00183760-8 displayed the lowest pollen viability values. At the HS conditions, the maximum pollen viability values were observed in genotypes BRA 00167017-3, BRA 00183755-8, and BRA 00167028-0. However, the genotypes BRA 00167447-2, BRA00183760-8, BRA00167007-4, and BRA00167420-9 demonstrated lower pollen viability at the HS conditions.

The heat susceptibility (*HSIpv*) is a useful criterion to select heat-tolerant genotypes[40]. The genotypes with *HSIpv* < 0.5 were considered as heat tolerant, *HSIpv* 0.5 – 0.99 were considered as moderately tolerant, and genotypes with *HSIpv* > 1.0 were considered as susceptible[40]. The genotypes categorized as tolerant were BRA00183760-8 (0.06), BRA 00167017-3 (0.36), BRA 00183755-8 (0.66) and BRA 00167028-0 (1.01). While BRA 00167447-2 (1.57), BRA00167420-9 (1.59), and BRA00167007-4 (1.89) were moderately tolerant (Table 4).

3.3. Heritability for Pollen Viability

The estimated heritability for pollen viability varied from 58.82% to 90.79%. The lowest h^2 was in BRA00167007-4 (58.82%) and the highest value was observed in BRA 00167023-1 (90.79%), followed by BRA 00183755-8 (91.32%), BRA 00167028-0 (88.56%), BRA 00167017-3 (88.35%), BRA00167420-9 (87.72%), BRA00183760-8 (83.82%) and BRA 00167447-2 (83.16%). All genotypes were classified as high heritability. Heritability is classified as low (<30%), medium (30 – 60%), and high (>60%)[42,44]. High heritability indicates that the selection for the trait is effective and is less influenced by environmental effects[42,44]. Broad-sense heritability becomes particularly intriguing when it applies to situations where the full genetic variability can be harnessed in clones or single-cross hybrids[45].

Table 4. Pollen viability (%) of the wild potatoes genotypes (*Solanum* sect. *Petota*, Solanaceae) from Embrapa Potato Genebank under control (CT) and heat stress (HS) conditions.

			Temperature treatment				Heritability (h^2) %
Genotype	Genesys code	Species	CT	HS	HSIpv	Score	

 doi:10.20944/preprints202311.0176.v1

BGB447	BRA 00183755-8	*S. malmeanum*	100 a*	91.5 abc	0.66	Tolerant	91.32
BGB100	BRA 00167017-3	*S. chacoense*	98.5 a	94.0 ab	0.36	Tolerant	88.35
BGB110	BRA 00167028-0	*S. chacoense*	96.0 a	83.5 abcd	1.01	Tolerant	88.56
BGB095	BRA 00167447-2	*S. chacoense*	89.5 abcd	71.5 bcde	1.57	Moderaltely tolerant	83.16
BGB001	BRA 00167007-4	*S. commersonii*	86.5 abcd	65.5 de	1.89	Moderaltely tolerant	58.82
BGB453	BRA 00183760-8	*S. commersonii*	69.0 b	69.5 bc	0.06	Tolerant	83.82
BGB068	BRA 00167420-9	*S. commersonii*	68.5 cde	54.5 e	1.59	Moderaltely tolerant	87.72

* Significant differences determined by Tukey's test ($P<0.05$) are indicated by average values labeled with different letters. These letters are represented in lowercase within the column when comparing genotypes within the same treatment.

Non-significant variation in pollen viability was observed as the temperature increased, indicating that the genotypes maintained their performance under both treatments. Heat stress has been demonstrated to adversely affect or even inhibit pollen viability in a diverse range of agricultural crops, including corn (*Zea mays* L.)[46], wheat (*Triticum aestivum* L.)[47], rice (*Oryza sativa* L.)[48], tomato (*Solanum lycopersicum* L.)[49], and wild potatoes (*Solanum* sect. *Petota*)[50]. Similar to our findings, under elevated temperature stress conditions, Bamberg and collaborators[50] observed that the percentage of pollen viability above 45% was seen in genotypes of different wild potatoes, including: *S. stoloniferum* Schltdl. (cited as *S. fendleri* A. Gray, PI 275156 with 50%, PI 497998 with 56%, PI 498004 with 63%), *S. stoloniferum* Schltdl. (cited as *S. polytrichon* Rydb. (PI 255547 with 49%), *S. demissum* Lindl. (PI 160208 with 70% and PI 498232 with 50%) and *S. jamesii* Torr. (PI 458425 and PI 195190, both with 45%), *S. chacoense* (PI 320293 with 49%), *S. commersonii* (PI 243503 with 53%), *S. infundibuliforme* Phil. (PI 498351 with 68%), and *S. boliviense* Dunal in DC. (cited as *S. megistacrolobum* Bitter (PI 473133 with 47%). Except for the genotypes that did not produce pollen and did not bloom under heat stress, *S. chacoense* (BRA 00167023-1) and *S. tuberosum* (BRA 00167251-8), respectively, the genotypes of the species *S. chacoense* (BRA 00167017-3, BRA 00167028-0, BRA 00167447-2), *S. commersonii* (BRA00167007-4, BRA00183760-8, BRA00167420-9), and *S. malmeanum* (BRA 00183755-8) exhibited similar pollen viability results to the study by Bamberg and collaborators[50], with values higher than 54.5%.

3.4. Pollen Size

Pollen size was compared using a non-parametric Wilcoxon-Mann-Whitney statistical test. The average of pollen size was 20 µm in all wild potato genotypes. Pollen size in BRA00167007-4 was 20 µm at the CT and 22 µm at the HS. BRA00167420-9, BRA 00167447-2, BRA 00167017-3, BRA 00167028-0, BRA 00183755-8, BRA00183760-8 exhibited pollen with 20 µm in size from both treatments. BRA 00167023-1 produces pollen with 20 µm and BRA 00167251-8 with 24 µm in size at the CT, also these two genotypes did not produce pollen and did not bloom at the HS treatment, respectively (Figure 3).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 November 2023 doi:10.20944/preprints202311.0176.v1

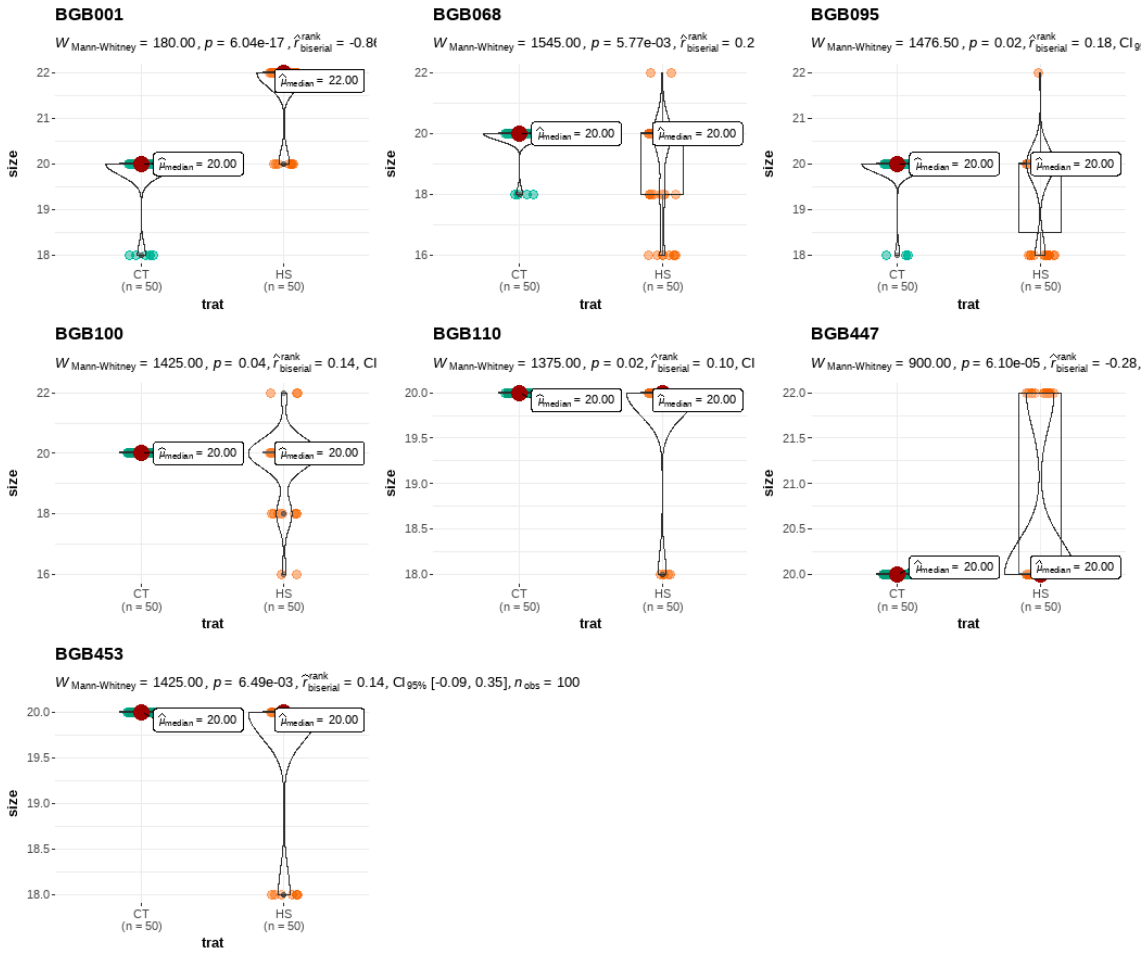

Figure 3. A non-parametric Wilcoxon-Mann-Whitney statistical test comparing pollen size from each genotype of wild potatoes (*Solanum* sect. *Petota*, Solanaceae) from Embrapa Potato Genebank at control (CT) and heat stress (HS) conditions, whereas in each accession (BGB code) the violin plot the frequency of each evaluation by dots, as the median.

The increase in temperature did not result in the production of non-reduced (2n) pollen grains in any of the genotypes evaluated in this study. To consider the production of 2n pollen grains, there needs to be 1.2 times increase compared to the normal[36].

The variations on pollen viability among the tested genotypes allows the selection of desirable genotypes for future potato crop improvement. This variation indicated that there is a way to identify promising genotypes based on the pollen viability trait. However, the PCV and GCV for all genotypes in both locations were moderate. A medium level of the coefficient of variation implies an equal influence of additive and nonadditive gene action[51]. In this present investigation, the PCV was higher than the GCV, but the differences between the PCV and GCV ranges were low, indicating the minimal impact of the environment on the expression of the traits, which is a sign of the heritable nature of the traits[51]. Several studies have also observed a higher PCV than GCV[52,53]. Hence, there is significant potential for precise trait selection based on the phenotypic expression[54–57].

Heritability is a powerful tool used to estimate the degree of variation within a population[58]. In this current investigation, a high heritability was observed for pollen viability. Previous research has consistently shown that high heritability implies minimal environmental influence on the genotype[59–61]. Therefore, minimized environmental effect on the studied trait allowed the high accuracy of 94% in the selection of genotypes under the HS conditions[40,62–64]. This information from the current study related to heritability is helpful for selecting the best trait for the improvement of crops[40,62–64]. The current study also revealed a high level of heritability for the pollen viability

 doi:10.20944/preprints202311.0176.v1

trait in potato wild genotypes. Nevertheless, placing exclusive reliance on heritability-based trait selection may not consistently lead to success, since broad-sense heritability accounts for the total genetic variance, which includes additive, dominant, and epistatic variances[65]. Therefore, estimation of the heritability of a group of genotypes coupled with high genetic advance is more reliable and efficient for the selection of desirable traits for a group of the population[66]. High heritability coupled with high genetic gain were found for the pollen viability in our study, which gave information to select superior genotypes[67,68]. Pollen viability is predominantly governed by additive gene action and can be improved by simple selection, hence, would be favorable for a potato breeding program[69].

Pollen viability studies in wild potato species grown under heat stress conditions are scarce in the literature. The identification of heat-tolerant wild potato genotypes regarding pollen viability favors the direction of future studies that relate stress, as plants respond to heat stress triggering a cascade of physiological, biochemical, and molecular processes and adapt by activating several stress-responsive genes[70,71]. Conducting a screening for the ability to produce viable pollen under heat stress conditions, as performed in this study, is crucial for identifying heat tolerance. It paves the way for further molecular characterization of genes involved in this process and facilitates the selection of promising genotypes for future stages of a potato genetic improvement program.

The abortion of pollen development observed in BRA 00167023-1 at the HS condition can be attributed to several factors, including the degradation of membrane integrity, the accumulation of reactive oxygen species (ROS), alterations in carbohydrate metabolism[72], disruptions in protein and lipid metabolism[73], and shifts in phospholipid profiles[74], which led to degeneration and abnormalities in tapetum cells[75].

Some causes for the non-development of the flowers in BRA 00167251-8 under heat stress conditions can be hypothesized. The transition to flowering and its timing are regulated by endogenous and environmental cues such as light and temperature[76]. Signaling pathways, in response to various endogenous cues such as hormones, the circadian clock, carbohydrate source-to-sink ratios, as well as autonomous and environmental stimuli like photoperiod and temperature, converge toward a limited number of floral integrator genes. Notably, these genes include *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)*, *FLOWERING LOCUS T (FT)*, and *AGAMOUS-LIKE 24 (AGL24)*[77]. These genes play a pivotal role in activating meristem identity genes, namely *LEAFY (LFY)*, *APETALA1 (AP1)*, *SEPALLATA3 (SEP3)*, and *FRUITFULL (FUL)*, which instigate the irreversible transition from a vegetative to a floral meristem[78] (Lohani et al. 2020).

To accomplish this goal, a detailed understanding of events underlying response to supra-optimal temperature during sexual reproduction and identification of reproductive traits related to thermotolerance will provide better tools for breeders to develop heat stress-resilient crops with enhanced crop productivity[79–87].

The identification of heat-tolerant wild potato genotypes that demonstrated enhance pollen viability is a significant milestone in the advancement of pre-breeding efforts aimed at improving the potato crop. Heat stress induces a cascade of physiological, biochemical, and molecular processes in plants, prompting them to activating numerous stress-responsive genes as a mean of adaptation[1,70]. The capacity to produce viable pollen under elevated temperatures, as evidenced in this study, plays a pivotal role in the identification of heat tolerance, and facilitates the subsequent molecular characterization of genes involved in this intricate process.

3. Conclusions

Pollen viability in wild potato germplasm is genetically variable whether grown at optimum temperature (control) and high temperature (stress).

The *S. malmeanum* genotype (BRA 00183755-8) and *S. chacoense* genotypes (BRA 00167017-3, BRA 00167028-0 and BRA00183760-8) were classified as tolerant for pollen viability under heat stress, signifying their potential as valuable candidates for future improvement studies.

Elevated temperatures do not lead to 2n pollen formation in the potato wild genotypes evaluated.

Acknowledgments: This work was supported by CAPES/PROAP, EMBRAPA, and FAPERGS (2551-0001703-0, 2019). RN acknowledges the CAPES (88887.622551/2021-00), The International Association for Plant Taxonomy for the IAPT 2022 Research Grants Program and Botanical Society of America for the BSA 2023 Research Grants Program. IB acknowledges CNPq (429368/2016-0) and its partnership with TWAS (TWAS/CNPq 154585/2017-3). GH acknowledges CNPQ (314590/2020-0) for the productivity research fellowship. The authors acknowledge USDA Potato Genebank for sending *S. chacoense* germplasm.

Conflicts Of Interest: The authors declare no conflict of interest.

References

1. Li, D.; Lu, X.; Zhu, Y.; Pan, J.; Zhou, S.; Zhang, X.; Zhu, G.; Shang, Y.; Huang, S.; Zhang, C. 2022. The multi-omics basis of potato heterosis. *Journal of Integrative Plant Biology* **2022**, *64:3*, 671-687.
2. Malagamba, P. Potato production from true seed in tropical climates. *HortScience* **1988**, *23:3*, 495-500.
3. Bloomfield, J.A.; Rose, T.J.; King, G.J. Sustainable harvest: managing plasticity for resilient crops. *Plant Biotechnology Journal* **2014**, *12*, 517–533.
4. IPCC: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty [Masson-Delmotte, V.; Zhai, P.; Pörtner, H. O.; Roberts, D.; Skea, J.; Shukla, P.R.; Pirani, A.; Moufouma-Okia, W.; Péan, C.; Pidcock, R.; Connors, S.; Matthews, J. B. R.; Chen, Y.; Zhou, X.; Gomis, M. I.; Lonnoy, E.; Maycock, T.; Tignor, M.; Waterfield, T. (eds.)]. **2018** *In Press*.
5. Pörtner, H.O.; Roberts, D.C.; Adams, H.; Adler, C.; Aldunce, P.; Ibrahim, Z.Z. Climate Change 2022: Impacts, Adaptation and Vulnerability. *IPCC: Geneva, Switzerland* **2022**.
6. Jarvis, A.; Lane, A.; Hijmans, R.J. The effect of climate change on crop wild relatives. *Agriculture, Ecosystem and Environment* **2008**, *126*, 13–23.
7. Vincent, H.; Amri, A.; Castañeda-Álvarez, N.P.; Dempewolf, H.; Dulloo, E.; Guarino, L.; Hole, D.; Mba, C.; Toledo, A.; Maxted, N. Modeling of crop wild relative species identifies areas globally for in situ conservation. *Communications Biology* **2019**, *2:1*, 1-8.
8. Silva, G.O.; Lopes, C.A. Sistema de produção da Batata. *Embrapa, Brasília* **2016**, 2.
9. Stokstad, E. The new potato. *Science* **2019**, *363:6427*, 574-577.
10. Hardigan, M.A.; Laimbeer, F.P.E; Newron, L.; Crisovan, E.; Hamilton, J.P.; Vaillancourt, B.; Wiegert-Rininger, K.; Wood, J.C.; Douches, D.S.; Farré, E.M.; Veilleux, R.E.; Nuell, C.R. Genome diversity of tuber-bearing *Solanum* uncovers complex evolutionary history and targets of domestication in the cultivated potato. *Proceedings of the National Academy of Sciences of the United States of America* **2017**, *114:46*, 9999–10008.
11. Spooner, D.M.; Ghislain, M.; Simon, R.; Jansky, S.H.; Gavrilenko, T. Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. *Botanical Review* **2014**, *80*, 283–383.
12. Hawkes, J.G.; Hjerting, J.P. The potatoes of Argentina, Brazil, Paraguay and Uruguay. A biosystematics study. *Oxford University Press, London* **1969**, pp. 525.
13. Hijmans, R.J.; Spooner, D.M. Geographic distribution of wild potato species. *American Journal of Botany* **2001**, *88*, 2101–2112.
14. Dempewolf, H.; Baute, G.; Anderson, J.; Kilian, B.; Smith, C.; Guarino, L. Past and future use of wild relatives in crop breeding. *Crop Science* **2017**, *57:3*, 1070-1082.
15. Warschefsky, E.; Penmetsa, R.V.; Cook, D.R.; Von Wettberg, E.J. Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. *American journal of Botany* **2014**, *101:10*, 1791-1800.
16. Jansky, S. Overcoming hybridization barriers in potato. *Plant Breeding* **2006**, *125:1*, 1–12.
17. Bethke, P.C.; Halterman, D.A.; Jansky, S. Are we getting better at using wild potato species in light of new tools?. *Crop Science* **2017**, *57:3*, 1241-1258.
18. Hawkes, J.G. Significance of wild species and primitive forms for potato breeding. *Euphytica* **1958**, *7:3*, 257–270.
19. Correll, D. The potato and its wild relatives. *Texas Research Foundation, Renner, USA* **1962**, pp. 606.
20. Lindhout, P.; Meijer, D.; Schotte, T.; Hutten, R.C.; Visser, R.G.; van Eck, H.J. Towards F1 hybrid seed potato breeding. *Potato Research* **2011**, *54:4*, 301-312.
21. Jansky, S.H.; Charkowski, A.O.; Douches, D.S.; Gusmini, G.; Richael, C.; Bethke, P.C.; Spooner, D.M.; Novy, R.G.; de Jong, H.; de Jong, W.S.; Bamberg, J.B.; Thompson, A.L.; Bizimungu, B.; Holm, D.G.; Brown, C.R.; Haynes, K.G.; Sathuvalli, V.R.; Veilleux, R.E.; Miller, J.C.; Bradeen, J.M.; Jiang, J. Reinventing potato as a diploid inbred line–based crop. *Crop Science* **2016**, *56*, 1412–1422.
22. Bradshaw, J.E. Breeding diploid F1 hybrid potatoes for propagation from botanical seed (TPS): comparisons with theory and other crops. *Plants* **2022**,*11:9*, 1121.
23. Endelman, J.B. Jansky, S.H. Genetic mapping with an inbred line-derived F2 population in potato. *Theoretical and Applied Genetics* **2016**, *129*, 935-943.

24. Meijer, D.; Viquez-Zamora, M.; Van Eck, H.J.; Hutten, R.C.B.; Su, Y.; Rothengatter, R.; Visser, R.G.F.; Lindhout, W.H.; Van Heusden, A.W. QTL mapping in diploid potato by using selfed progenies of the cross *S. tuberosum*× *S. chacoense*. *Euphytica* **2018**, *214*, 1-18.
25. Song, L.; Endelman, J.B. Using haplotype and QTL analysis to fix favorable alleles in diploid potato breeding. *The Plant Genome* **2023**, e20339.
26. De Vries, M.; ter Maat, M.; Lindhout, P. The potential of hybrid potato for East-Africa. *Open Agriculture* **2016**, *1:1*, 151-156.
27. Bethke, P.C.; Halterman, D.A.; Francis, D.M.; Jiang, J.; Douches, D.S.; Charkowski, A.O.; Parsons, J. Diploid potatoes as a catalyst for change in the potato industry. American *Journal of Potato Research* **2022**, *99:5-6*, 337-357.
28. Bethke, P.C.; Halterman, D.A.; Jansky, S.H. Potato germplasm enhancement enters the genomics era. *Agronomy* **2019**, *9:10*, 575.
29. Watson, A.; Ghosh, S.; Williams, M.J.; Cuddy, W.S.; Simmonds, J.; Rey, M.D.; Hatta, M.A.M.; Hinchliffe, A.; Steed, A.; Reynolds, D.; Adamsky, N.M.; Breakspear, A.; Korolev, A.; Rayuner, T.; Dixon, L.E.; Riaz, A.; Martin, W.; Ryan, M.; Edward, D.; Batley, J.; Raman, H.; Carter, J.; Rogers, C.; Domoney, C.; Moore, G.; Harwood, W.; Nicholson, P.; Dieters, M.J.; DeLacy, I.H.; Zhou, J.; Uauy, C.; Boden, S.A.; Park, R.F.; Wulff, B.B.H.; Hickey, L.T. Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature Plants* **2018**, *4:1*, 23-29.
30. Schindfessel, C.; De Storme, N.; Trinh, H.K.;Geelen, D. Asynapsis and meiotic restitution in tomato male meiosis induced by heat stress. Frontiers in *Plant Science* **2023**, 14.
31. Omidi, M.; Siahpoosh, M.R.; Mamghani, R.; Modarresi, M. The influence of terminal heat stress on meiosis abnormalities in pollen mother cells of wheat. *Cytologia* **2014**, *79:1*, 49-58.
32. Alam, M.A.; Seetharam, K.; Zaidi, P.H.; Dinesh, A.; Vinayan, M.T.; Nath, U.K. Dissecting heat stress tolerance in tropical maize (*Zea mays* L.). *Field Crops Research* **2017**, *204*, 110-119.
33. Zhou, Q.; Cheng, X.; Kong, B.; Zhao, Y.; Li, Z.; Sang, Y.; Wu, J.; Zhang, P. Heat shock-induced failure of meiosis I to meiosis II transition leads to 2n pollen formation in a woody plant. *Plant Physiology* **2022**, *189:4*, 2110-2127.
34. Alexander, M.P. Differential staining of aborted and non-aborted pollen. *Stain Technology* **1969**, *44*, 117–122.
35. Marks, G.E. An aceto-carmine glycerol jelly for use in pollen-fertility counts. *Stain Technology* **1954**, *29:5*, 277-277.
36. Ordoñez, B.; Orrillo, M.; Bonierbale, M.W. Technical manual potato reproductive and cytological biology. *International Potato Center (CIP)* **2017**, pp.69.
37. Ferreira, E.B.; Cavalcanti, P.P.; Nogueira, D.A. ExpDes.pt: Experimental Designs package (Portuguese). *R package version 1.1.2.*, **2021**. Available online: < https://cran.utstat.utoronto.ca/web/packages/ExpDes.pt/ExpDes.pt.pdf> (01/08/2023)
38. Rstudio Team Rstudio: Integrated Development for R. Rstudio, PBC, Boston, MA, 2020. Available online: http://www.rstudio.com/. (01/08/2023).
39. Fischer, R.A.; Maurer, R. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research* **1978**, *29:5*, 897-912.
40. Khan, I.; Wu, J.; Sajjad, M. Pollen viability-based heat susceptibility index (HSIpv): A useful selection criterion for heat-tolerant genotypes in wheat. *Frontiers in Plant Science* **2022**, *13*, 1064569.
41. Burton, G.W. Quantitative inheritance in grasses. *In Proceedings of the 6th International Grassland Congress, State College, PA, USA* **1952**, *17–23*, pp. 277–283.
42. Allard, R.W. Principles of plant breeding. *John Willey and Sons. Inc. New York* **1960**, pp. 485.
43. Robinson, H.F.; Cornstock, R.E.; Harvey, P.H. Estimates of heritability and degree of dominance in corn. *Agronomy Journal* **1949**, *41*, 353–359.
44. Johnson, H.W.; Robinson, H.F.; Comstock, R.E. Estimation of genetic and environmental variability in soybean. *Agronomy Journal* **1955**, *47*, 314-318.
45. Bernardo, R. Breeding for quantitative traits in plants. 2nd ed. *Woodbury: Stemma Press 2010*, pp. 390.
46. Begcy, K.; Nosenko, T.; Zhou, L.Z.; Fragner, L.; Weckwerth, W.; Dresselhaus, T. Male sterility in maize after transient heat stress during the tetrad stage of pollen development. *Plant Physiology* **2019**, *181:2*, 683-700.
47. Ullah, A.; Nadeem, F.; Nawaz, A.; Siddique, K.H.; Farooq, M. Heat stress effects on the reproductive physiology and yield of wheat. *Journal of Agronomy and Crop Science* **2022**, *208:1*, 1-17.
48. Kumar, N.; Kumar, N.; Shukla, A.; Shankhdhar, S.C.; Shankhdhar, D. Impact of terminal heat stress on pollen viability and yield attributes of rice (*Oryza sativa* L.). *Cereal Research Communications* **2015**, *43:4*, 616-626.
49. Paupière, M.J.; Müller, F.; Li, H.; Rieu, I.; Tikunov, Y.M.; Visser, R.G.; Bovy, A.G. Untargeted metabolomic analysis of tomato pollen development and heat stress response. *Plant Reproduction* **2017**, *30:2*, 81-94.
50. Bamberg, J.B. Screening potato (*Solanum*) species for male fertility under heat stress. *American Potato Journal* **1995**,*72:1*, 23-33.

51. Allard, R.W.; Hansche, P.E. Some parameters of population variability and their implications in plant breeding. *Advances in Agronomy* **1964**, *16*, 281-325.
52. Nagalakshmi, R.M.; Ravikesavan, R.; Paranidharan, V.; Manivannan, N.; Firoz, H.; Vignesh, M.; Senthil, N. Genetic variability, heritability and genetic advance studies in backcross populations of maize (*Zea mays* L.). *Electronic Journal of Biotechnology* **2018**, *9*, 1137–1145.
53. Ganesan, K.N.; Nallathambi, G.; Senthil, N.; Tamilarasi, P.M. Genetic divergence (*Zea mays* L.) Analysis in indigenous maize. *Electronic Journal of Biotechnology* **2010**, *1*, 1241–1243.
54. Schoper, J.B.; Lambert, R.J.; Vasilas, B.L. Maize pollen viability and ear receptivity under water and high temperature stress 1. *Crop Science* **1986**, *26:5*, 1029-1033.
55. Panthee, D.R.; Kressin, J.P.; Piotrowski, A. Heritability of flower number and fruit set under heat stress in tomato. *HortScience* **2018**, *53:9*, 1294-1299.
56. Hazra, P.; Ansary, S.H., Genetics of heat tolerance for floral and fruit set to high temperature stress in tomato (*Lycopersicon esculentum* Mill.). *SABRAO Journal of Breeding & Genetics* **2008**, *40*(2).
57. Meena, R.K.; Kumar, S. Variability, Heritability and Genetic Advance in Tomato (*Solanum lycopersicum* L.) Genotypes. *International Journal of Plant & Soil Science* **2023**, *35:4*, 138-144.
58. Visscher, P.; Hill, W.; Wray, N. Heritability in the genomics era — concepts and misconceptions. *Nature Review Genetics* **2008**, *9*, 255–266.
59. Petr, F.C.; Frey, K.J. Genotypic Correlations, Dominance, and Heritability of Quantitative Characters in Oats 1. *Crop Science* **1966**, *6:3*, 259-262.
60. Chatara, T.; Musvosvi, C.; Houdegbe, A.C.; Sibiya, J. Variance Components, Correlation and Path Coefficient Analysis of Morpho-Physiological and Yield Related Traits in Spider Plant (*Gynandropsis gynandra* (L.) Briq.) under Water-Stress Conditions. *Agronomy* **2023**, *13:3*, 752.
61. Lal, N.; Singh, A.; Kumar, A.; Pandey, S. Assessment of Variability, Correlation and Path Analysis for the Selection of Elite Clones in Litchi Based on Certain Traits. *Erwerbs-Obstbau* **2023**, *65*,1747–1754
62. Olakojo, S.A.; Olaoye, G. Correlation and heritability estimates of maize agronomic traits for yield improvement and *Striga asiatica* (L.) kuntze tolerance. *African Journal of Plant Science* **2011**, *5*, 365–369.
63. Aminu, D.; Izge, A.U. Heritability and correlation estimates in maize (*Zea mays* L.) Under drought conditions in northern guinea and sudan savannas of Nigeria. *World Journal of Agricultural Sciences* **2012**, *8*, 598–602.
64. Azam, M.G.; Sarker, U.; Maniruzzaman, M.; Banik, B.R. Genetic variability of yield and its contributing characters on CIMMYT maize inbreds under drought stress. *Bangladesh Journal of Agricultural Research* **2015**, *39*, 419–426.
65. Hill, W.G.; Goddard, M.E. Visscher, P.M. Data and theory point to mainly additive genetic variance for complex traits. *PloS Genetics* **2008**, *4:2*, e1000008.
66. Prakash, R.; Ravikesavan, R.; Vinodhana, N.K.; Senthil, A. Genetic variability, character association and path analysis for yield and yield component traits in maize (*Zea mays* L.). *Electronic Journal of Biotechnology* **2019**, *10*, 518–524.
67. Divakara, B.N.; Upadhyaya, H.D.; Wani, S.P.; Gowda, C.L. Biology and genetic improvement of Jatropha curcas L.: a review. *Applied Energy* **2010**, *87:3*, 732-742.
68. Kalloo, G. Genetic improvement of tomato. *Springer Science & Business Media* **2012**, *14*.
69. Dane, F.; Hunter, A.G.; Chambliss, O. L. Fruit set, pollen fertility, and combining ability of selected tomato genotypes under high-temperature field conditions. *Journal of the American Society for Horticultural Science* **1991**, *116:5*, 906-910.
70. Hasanuzzaman, M.; Nahar, K.; Alam, M.; Roychowdhury, R.; Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences* **2013**, *14:5*, 9643-9684.
71. Paupière, M.J.; Van Heusden, A.W.; Bovy, A.G. The metabolic basis of pollen 12hermos-tolerance: perspectives for breeding. *Metabolites* **2014**, *4:4*, 889-920.
72. Liu, Y.; Li, J.; Zhu, Y.; Jones, A.; Rose, R.J.; Song, Y. Heat stress in legume seed setting: effects, causes, and future prospects. *Frontiers in Plant Science* **2019**, *10*, 938.
73. Narayanan, S.; Prasad, P.V.V.; Welti, R. Alterations in wheat pollen lipidome during high day and night temperature stress. *Plant, Cell & Environment* **2018**, *41*, 1749–1761.
74. Djanaguiraman, M.; Perumal, R.; Ciampitti, I.A.; Gupta, S.K.; Prasad, P.V.V. Quantifying pearl millet response to high temperature stress: thresholds, sensitive stages, genetic variability and relative sensitivity of pollen and pistil. *Plant, Cell & Environment* **2018**, *41*, 993–1007.
75. Cecchetti, V.; Celebrin, D.; Napoli, N.; Ghelli, R.; Brunetti, P.; Costantino, P.; Cardarelli, M. An auxin maximum in the middle layer controls stamen development and pollen maturation in Arabidopsis. *New Phytologist* **2013,**1194–1207.
76. Araki T. Transition from vegetative to reproductive phase. *Current Opinion in Plant Biology* **2001**, *4*, 63–68.
77. Blümel, M.; Dally, N.; Jung C. Flowering time regulation in crops—what did we learn from Arabidopsis? *Current Opinion in Biotechnology* **2015**, *32*, 121–129.

78. Lohani, N.; Singh, M.B.; Bhalla, P.L. High temperature susceptibility of sexual reproduction in crop plants. *Journal of Experimental Botany* **2020**, *71*, 555–568.
79. Aiqing, S.; Somayanda, I.; Sebastian, S.V.; Singhm, K.; Gill, K.; Prasad, P.V.; Jagadish, S.V. Heat stress during flowering affects time of day of flowering, seed set, and grain quality in spring wheat. *Crop Science* **2018**, *58:1*, 380–392.
80. Chaturvedi, P.; Wiese, A.J.; Ghatak, A.; Zaveska D.L.; Weckwerth, W.; Honys, D. Heat stress response mechanisms in pollen development. *New Phytologist* **2021**, *231:2*, 571-585.
81. Delph, L.F.; Johannsson, M.H.; Stephenson, A.G. How environmental factors affect pollen performance ecological and evolutionary perspectives. *Ecology* **1997**, *78*, 1632- 1639.
82. Djanaguiraman, M.; Prasad, P.V.; Boyle, D.; Schapaugh, W. Soybean pollen anatomy, viability and pod set under high temperature stress. *Journal of Agronomy and Crop Science* **2013**, *199*, 171–177.
83. Djanaguiraman, M.; Prasad, P.V.; Murugan, M.; Perumal, R.; Reddy, U.K. Physiological differences among sorghum (*Sorghum bicolor* L. Moench) genotypes under high temperature stress. *Environmental and Experimental Botany* **2014**, *100*, 43–54.
84. Endo, M.; Tsuchiya, T.; Hamada, K.; Kawamura, S.; Yano, K.; Ohshima, M.; Higashitani, A.; Watanabe, M.; Kawagishi-Kobayashi, M. High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant & Cell Physiology* **2009**, *50*, 1911–1922.
85. Jain, M.; Chourey, P.S.; Boote, K.J.; Allen, L.H.Jr. Short-term high temperature growth conditions during vegetative-to-reproductive phase transition irreversibly compromise cell wall invertase-mediated sucrose catalysis and microspore meiosis in grain sorghum (*Sorghum bicolor*). *Journal of Plant Physiology* **2010**, *167*, 578–582.
86. Pécrix, Y.; Rallo, G.; Folzer, H.; Cigna, M.; Gudin, S.; Le Bris, M. Polyploidization mechanisms: temperature environment can induce diploid gamete formation in *Rosa* sp. *Journal of Experimental Botany* **2011**, *62*, 3587–3597.
87. Razzaq, M.K.; Rauf, S.; Khurshid, M.; Iqbal, S.; Bhat, J.A.; Farzand, A.; Riaz, A.; Xing, G.; Gai, J.,Pollen Viability an Index of Abiotic Stresses Tolerance and Methods for the Improved Pollen Viability. *Pakistan Journal of Agricultural Research* **2019**,*32:4*, 609-624.