

Integrating Phenotypic Selection, Population Structure, and SNP-Based Genetic Diversity to Improve Heat Tolerance in Spring Wheat

Huda Ali Sleem

Sohag University

Alaa A. Said

Sohag University

Ahmed R. M. Ridwan

Sohag University

Ahmed Sallam

amsallam@aun.edu.eg

Badr University in Assiut

Research Article

Keywords: High temperature, abiotic stress tolerance, genetic variability, heritability; correlation analysis, genotype selection, genetic diversity, wheat breeding

Posted Date: September 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10913738/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Heat stress is a major abiotic constraint limiting wheat production across its different growth stages, as it drastically impairs growth and reduces grain yield. A set of 103 highly diverse wheat genotypes was evaluated under normal (N) and heat stress (HS) conditions. The measured traits included plant height (PH), days to heading (DTH), days to maturity (DTM), grain number per spike (GNPS), grain yield per spike (GYPS), thousand-kernel weight (TKW), spike length (SL), and number of spikelets per spike (NSPS). The analysis of variance showed high genetic variation among genotypes under both conditions. High heritability estimates were observed for all traits in each condition. Grain yield per spike (GYPS) was the trait most affected by heat stress, with reductions of 57.8% and 46.6% in the first and second seasons, respectively, whereas spike length (SL) was the least affected trait, with reductions of 11.3% and 10.9% in the first and second seasons, respectively. Grain yield per spike (GYPS) showed a strong negative correlation with days to heading (DTH) and days to maturity (DTM), and a strong positive correlation with grain number per spike (GNPS), number of spikelets per spike (NSPS), and thousand-kernel weight (TKW) across all four environments. Selection results revealed 11 consistently low-yielding genotypes, while 13 high-yielding genotypes repeatedly ranked among the top 20 in both seasons. The genetic distance among the selected genotypes ranged from 0.236 to 0.627, with the greatest distance between WAS_140 (Morocco) and WAS_160 (Saudi Arabia) and the smallest between WAS_139 and WAS_140 (both from Morocco). The genotype WAS_016 (Egypt) was genetically distinct from the other selected genotypes. In breeding programs, crossbreeding between genotypes WAS_160 and WAS_140 appears promising for developing heat-tolerant, high-yielding lines.

Introduction

Wheat is one of the most important food crops worldwide. However, despite its crucial role in global food security, wheat production is increasingly constrained by heat stress, an abiotic stress factor that is being exacerbated by the ongoing increase in the frequency and intensity of hot and dry weather conditions [1]. According to FAO[2] the global wheat harvested area reached 219.5 million hectares, with an average yield of 3,637.4 kg /ha and total production of 798.4 million metric tons. Rising temperatures, particularly during sensitive developmental stages such as flowering and grain filling, adversely affect wheat productivity by reducing grain size, grain number, and ultimately overall grain yield[3] .

Wheat yields are predicted to decrease by 6% for all temperature degrees of temperature rise due to climate change[4]. Climate change is negatively impacting wheat yields due to the adverse effects of rising temperatures on reproductive physiology. Wheat is affected by heat stress at various growth stages, but it is particularly sensitive to high temperatures during the flowering and reproductive phases. Heat stress limits the transfer of resources to growing grains during anthesis, leading to small grains and low yields [5]. The two main abiotic stresses limiting wheat yield are heat and drought. Drought stress disturbs vital physiological and biochemical processes, grain quantity, photosynthetic activity, chlorophyll concentration, and endosperm starch synthesis [6]. Late sowing reduced spike length by 13%, grain number per spike by 10%, and 1000-kernel weight by 13% compared with timely sowing[4] .Expanding wheat cultivation into new habitats is necessary to provide food security due to limited agricultural land. However, abiotic issues including heat, salinity, and water shortages are present in these new habitats. Thus, ensuring steady grain output is one of the main goals of breeding efforts, especially in subtropical areas[7].

Heat stress in wheat can be assessed in both controlled environments and field trials. Field experiments expose wheat to natural heat episodes and other environmental factors, making them more informative for grain yield and adaptation. Evidence from wheat research shows that heat tolerance involves strong genotype-by-environment interactions, and that both field testing and controlled-environment phenotyping contribute valuable, complementary information. Thus, using both approaches together is an efficient strategy, as it connects physiological responses with agronomic performance. Under field conditions in Egypt, heat stress was applied before on wheat, barley crop to investigate its effects on yield attributes by sowing the genotypes one month later (late of December) than the optimum conditions (mid - November), so the genotypes are exposed to the high waves of heat temperature at heading, anthesis and grain-filling stages compared to those planted in the optimum sowing date

Breeding programs that use direct selection for yield in plants may be deceptive. However, breeding program take much time to achieve their goals. Information about the genetic diversity and correlation between agronomic parameters and grain yield are essential for successful selection [8]. Two of the most important elements in the development of many crop plants are genetic stability and diversity. Selecting a high-yielding genotype with broad adaptation is a significant issue for plant breeders[9]

Analyzing genetic diversity among selected genotypes (e.g. heat tolerant) using molecular markers such as single-nucleotide polymorphism (SNP) markers enables the estimation of genetic distance among genotypes. This information helps identify genetically distant parents for hybridization, which may increase the likelihood of obtaining superior hybrids more accurately and efficiently than traditional breeding methods.

In this study, wheat genotypes originating from different countries were evaluated to identify superior performers under heat-stress conditions based on multiple traits considered jointly. A subset of 13 heat-tolerant genotypes was selected using a multi-trait selection approach, and their genetic distances were estimated to characterize their genetic divergence. The specific objectives were to (i) identify high-performing wheat genotypes under heat stress from a geographically diverse panel using combined multi-trait criteria, and (ii) assess genetic distance among the selected genotypes to facilitate the choice of genetically divergent parents for crossing and the development of improved hybrids.

Materials and methods

Plant material:

A collection of 103 spring wheat genotypes has high diversity in morphological and yield traits. These genotypes were obtained from the global core collection of the USDA-ARS. These genotypes were collected from 14 different countries, as shown in Supplementary Table S1. Seventeen of the 103 genotypes are local and typically grown in Egypt.

Experimental Layout

All the genotypes were planted at the farm of the Faculty of Agriculture, Sohag University, Egypt (26.4706°N, 31.6725°E; <https://power.larc.nasa.gov/data-access-viewer/>), where the soil was sandy loam, as shown in Supplementary Table S2.

All genotypes were grown under two conditions across two growing seasons: normal (N) and heat stress (HS). In both growing seasons, heat stress was imposed by delaying sowing by one month, exposing plants to high temperatures during the heading, anthesis, and grain-filling stages, which occurred in March and April [10] In the 2023/2024 and 2024/2025 seasons, genotypes were sown on November 27 and December 5 under normal conditions, respectively. Under heat stress, all genotypes were sown on December 27 and December 31, respectively. Each genotype was planted in three replications. Each plot contained 10 rows, with each row representing a different genotype. Grains of each genotype were sown in a single 1 m row, with 10 cm between plants within a row and 20 cm between rows. During the growing season, all planted genotypes were grown in sandy loam soil and irrigated 15 times under normal conditions and 11 times under heat-stress conditions

The maximum temperatures for the two seasons under both normal and heat-stress conditions are shown in Figs. 1a and 1b. In the first season, maximum temperatures ranged from 22.4 to 43°C, while in the second season, they ranged from 22.7 to 39°C. In both seasons, maximum temperatures in April exceeded 30°C and coincided with the onset of heading under heat stress (35.9°C in season 1 and 34.2°C in season 2). Under normal conditions, heading commenced at maximum temperatures of 22.4°C (season 1) and 24.9°C (season 2). Under both conditions, the heading period was shorter in the first season than in the second. Supplementary Table S3 displays the average temperatures for both seasons. <https://power.larc.nasa.gov/data-access-viewer/>

Trait scoring:

Under two conditions during two seasons, eight traits of each genotype were measured.

- Days to heading (DTH; days) was scored as the number of days from sowing to the date when 50% of the plants started heading.
- Days to maturity (DTM; days): number of days from sowing until 50% of the plants showed signs of maturity.
- Plant height (PH; cm): measured from the ground to the tip of the main spike.
- Grain number per spike (GNPS),
- Grain yield per spike (GYPS; g),
- Thousand kernel weight (TKW; g),
- Main spike length (SL; cm),
- Number of spikelets per spike (NSPS).

Moreover, the reduction due to heat stress (RDH) for each trait across the two seasons was calculated as follows:

$$RDH = \frac{X_N - X_{HS}}{X_N} \times 100$$

where RDH is the percentage reduction in the trait due to heat stress, X_N is the mean value of all genotypes for the respective trait under normal conditions, and X_{HS} is the mean value of the same trait under heat stress, calculated separately for each growing season (2023/2024 and 2024/2025).

Statistical analysis:

Combined analysis of variance for phenotypic data was performed using PLABSTAT software[11] through the following model: $Y_{iknj} = \mu + y_k + t_n + g_i + r_{j(k)} + (yg)_{ki} + (yt)_{kn} + (tg)_{in} + (ygt)_{kin} + e_{iknj}$, where Y_{iknj} is the observation of genotype i in year k , treatment n , and replication j within year k ; μ is the general average; y_k , t_n , g_i , and $r_{j(k)}$ are the main effects of year, treatment, genotype, and replication nested within year, respectively. The terms $(yg)_{ki}$, $(yt)_{kn}$, $(tg)_{in}$, and $(ygt)_{kin}$ represent the corresponding interactions, and e_{iknj} is the residual error. Years, genotypes and replicates were considered random effects, and treatment was considered a fixed effect. All the graphics of measured traits in this study were plotted through the SR plot website <https://www.bioinformatics.com.cn/en> and R software[12] Heritability in the broad sense (H^2) % for each trait was calculated with the following equation: $H^2 = (\text{genotypic variance} / \text{phenotypic variance})$ using the GENOT command in PLABSTAT software. Phenotypic correlation among traits under each condition and each growing season was computed by PLABSTAT software.

Selection index for heat tolerance:

The analysis of the selection index (SI) for spike traits was performed in R software using the "metan" package[13] First, genetic and phenotypic parameters were estimated using the gamem () function. Then, the index was computed using the Smith–Hazel selection index [14, 15] Spike traits (GNPS, GYPS, TKW, SL, and NSPS) held the same economic importance, and equal economic weights were assigned to each trait. To account for differing trait scales, we recalculated the Smith–Hazel index using standardized economic weights, obtained by dividing each target weight (1.0) by the trait's phenotypic standard deviation. A selection of intensity was 19.4 % applied to identify the highest- and lowest-performing genotypes. In each year, the 20 genotypes with the highest selection index (SI) were selected. Venn diagrams (the Venny tool) (<https://bioinfo.gp.cnb.csic.es/tools/venny/>) were then used to identify genotypes that were common across the two seasons under heat stress conditions.

Genetic analysis:

The 103 genotypes were previously genotyped using genotyping-by-sequencing (GBS) by Mourad et. al [16], who extensively investigated their genetic diversity. Population structure analysis was based on 11,362 single-nucleotide polymorphisms (SNP), classified the genotypes into three subpopulations (SPs): SP1(48 genotypes), SP2 (46 genotypes), and SP3 (nine genotypes). In the present study, the performance of genotypes in each SP was compared under heat stress.

Moreover, genetic distance among the selected genotypes was assessed using the same set of 11,362 SNP markers. Genetic distances were calculated in R software [12] using the ade4 package [17] to identify the most genetically divergent genotypes for use as potential parents in future breeding programs.

The population structure was previously analyzed using genotyping-by-sequencing (GBS) SNP markers to determine genetic relationships and the number of subpopulations among the 103 genotypes. The genotypes were grouped into three subpopulations containing 48, 46, and 9 genotypes, respectively GBS produced 11,362 filtered SNP markers, which were used to calculate genetic distances among 13 high-yielding genotypes. SNP data were converted into numerical format using Tassel 5 software [18] and subsequently analyzed in (R version 4.5.2) software [12] with the ade4 package [17] to compute genetic distances among the selected genotypes. WAS_140 (Morocco) and WAS_160 (Saudi Arabia) exhibited high genetic distance, while WAS_139 (Morocco) and WAS_140 (Morocco) exhibited low genetic distance.

Results

The analysis of variance (ANOVA) for all traits under different environmental conditions.

The analysis of variance (ANOVA) for all traits under normal and heat stress conditions during the 2023/2024 and 2024/2025 growing seasons is presented in Table 1. The results of the combined ANOVA showed highly significant differences between the years (Y) in all measured traits. Highly significant differences between the two treatments for all traits except for plant height (PH), number of spikelets per spike (NSPS). The differences among genotypes (G) in all measured traits in both seasons were highly significant. The G×T interaction was highly significant for all traits in both growing seasons. Similarly, the G ×Y interaction was highly significant for all traits in both growing seasons. The heritability estimates ranged from 0.72 (PH) to 0.96 (DTH). The evaluated genotypes under normal (N) and heat stress (HS) conditions are presented for the 2023/2024 and 2024/2025 seasons in Table 2. The mean performance of the genotypes under normal conditions was higher than under heat stress for all measured traits during the two seasons. Notably, the mean performance of the genotypes for all traits in the first season was higher than in the second season under normal conditions, while there was instability in the performance of the genotypes for all traits except grain yield per spike, which showed similar performance across the two seasons under heat stress conditions.

Table 1
Analysis variance for measured traits under both conditions in two seasons.

S.O. V	df	PH	DTH	DTM	GNPS	GYPS	TKW	SL	NSPS
Year (Y)	1	3899.77**	3462.83**	6994.33**	3136.06**	3130.99**	3588.05**	717.46**	1362.98**
Treatments (T)	1	0.01	6478.42**	5828.29**	99.55**	111.32**	151.21**	137.76**	0.71
Genotypes (G)	102	3.61**	30.20**	17.21**	6.21**	4.85**	4.87**	27.78**	4.73**
G*T	102	5.40**	24.76**	9.97**	2.82**	5.41**	5.05**	7.17**	4.44**
G*Y	102	8.87**	15.20**	4.32**	12.23**	9.94**	9.73**	3.92**	6.44**
H ² %		72.3	96.7	94.2	83.9	79.4	79.5	96.4	78.9
PH; plant height, DTH; day to heading, DTM; day to maturity, GNPS; grain number per spike, GYPS; grain yield per spike, TKW; thousand kernel weight, SL; spike length, NSPS; number of spikelets per spike, df; degree of freedom, H ² %; broad sense heritability									

Table 2
Mean, Maximum (Max) and Minimum (Min) for traits under normal conditions (N) and heat stress (HS) in two seasons.

Season 2023/2024									
Trait		PH	DTH	DTM	GNPS	GYPS	TKW	SL	NSPS
Mean	N	122.4	100.1	138.7	46.6	1.8	41.9	11.9	19.2
	HS	91	92.5	91.7	27.4	0.8	29.6	10.5	14.9
Max	N	153.9	132.3	157.7	98.1	4.4	66.2	20.8	25.3
	HS	128	116.7	112.7	64.3	2.8	49.5	18.9	21.8
Min	N	83.2	75.7	120.7	17.9	0.5	22.4	5.4	11.3
	HS	63.8	70.6	70.7	3	0.1	10	4.9	6.8
L.S.D 5%	N	7.4	2.03	1.62	7.72	0.44	5.04	1.14	1.96
	HS	10.65	2.21	1.37	5.86	0.29	6.09	1.28	1.87
Season 2024/2025									
Mean	N	116.5	89.9	121.1	40.6	1.5	39.3	11.2	18.5
	HS	96.9	82.9	108.7	27.6	0.8	27.1	9.9	15.8
Max	N	147.3	129	146.7	92.5	3.7	63	19.9	25
	HS	132	115.7	132.7	61.5	2.1	47.3	19.8	24
Min	N	81.8	60.5	96.7	6	0.2	15.6	4.7	11
	HS	74	57.7	95.3	1	0.1	10	4.6	6.7
L.S.D5%	N	6.52	2.07	1.99	6.25	0.26	4.38	1.07	2.13
	HS	5.15	1.98	1.31	4.96	0.27	4.45	1.02	2.73
PH; plant height, DTH; day to heading, DTM; day to maturity, GNPS; grain number per spike, GYPS; grain yield per spike, TKW; thousand kernel weight, SL; spike length, NSPS; number of spikelets per spike, L.S.D; least significant difference at level 5%									

Effect of heat stress on all measured traits.

The reduction in each trait due to heat stress for all measured traits during the 2023/2024 and 2024/2025 seasons is presented in Fig. 2. Under heat stress in both seasons, all traits decreased due to heat stress. The reduction due to heat stress in the first season was higher than in the second season for all measured traits except DTH and TKW. Heat stress had a similar effect on days to heading, as genotypes headed earlier under heat stress than under normal conditions, with reductions of 7.6% and 7.7% in the first and second seasons, respectively. The highest was observed for GYPS, with 57.8 and 46.6% reduction due to heat stress in first and second seasons, respectively. The lowest reduction, on the other hand, was found in the SL, with 11.3 (first season) and 10.9% (second season). All traits showed a difference in reduction due to heat stress between the two seasons, except for SL and DTH, which showed a similar reduction between the two seasons.

The phenotypic correlations among all traits under normal and heat stress conditions in two seasons.

The phenotypic correlation among all traits under normal conditions and heat stress is shown in Tables 3 and 4, respectively. Under normal and heat stress conditions, a high and significant positive correlation was found for each trait between the two growing seasons. DTH between the two growing seasons under each condition had the highest significant correlation with 0.89** (heat stress) and 0.91** (normal). In all four environments, GYPS was strongly positively correlated with GNPS, NSPS, and TKW, while it was significantly negatively correlated with DTH and DTM. Under the four environments, SL did not show consistent correlations with the other measured traits. Under heat stress conditions, spike index (SI) including GNPS, GYPS, TKW, SL, and NSPS, was significantly correlated with all yield-related traits except SL (in both seasons) and PH in the second season. In both seasons, SI showed high and negative correlation with DTH and DTM, while it has high and positive significant correlations with GNPS, GYPS, TKW, and NSPS. Under heat stress conditions. The spike index (SI) showed a highly positive correlation ($r = 0.85^{**}$) between the two growing seasons under heat stress conditions, as displayed in Fig. 3a.

Table 3
Correlation between traits under normal conditions for two seasons (Above, bold season 1, below season 2).

Traits	PH	DTH	DTM	GNPS	GYPS	TKW	SL	NSPS
PH	0.76 ^{***}	0.48^{***}	0.43^{***}	-0.10 ^{ns}	-0.09 ^{ns}	-0.28 ^{**}	0.18 ^{ns}	0.29 ^{**}
DTH	0.27 ^{**}	0.95 ^{***}	0.78^{***}	-0.48^{***}	-0.49^{***}	-0.53^{***}	0.23 [*]	-0.04 ^{ns}
DTM	0.23 [*]	0.95 ^{***}	0.78 ^{***}	-0.36^{***}	-0.36^{***}	-0.43^{***}	0.15 ^{ns}	0.09 ^{ns}
GNPS	-0.05 ^{ns}	-0.63 ^{***}	-0.58 ^{***}	0.88 ^{***}	0.82^{***}	0.36^{***}	-0.12 ^{ns}	0.55 ^{***}
GYPS	-0.18 ^{ns}	-0.63 ^{***}	-0.58 ^{***}	0.78 ^{***}	0.75 ^{***}	0.64^{***}	0.01 ^{ns}	0.46 ^{***}
TKW	-0.29 ^{**}	-0.60 ^{***}	-0.54 ^{***}	0.45 ^{***}	0.67 ^{***}	0.82 ^{***}	-0.09 ^{ns}	0.05 ^{ns}
SL	0.28 ^{**}	0.34 ^{***}	0.37 ^{***}	-0.18 ^{ns}	-0.21 [*]	-0.14 ^{ns}	0.85 ^{***}	0.13 ^{ns}
NSPS	0.18 ^{ns}	-0.34 ^{***}	-0.30 ^{**}	0.68 ^{***}	0.51 ^{***}	0.15 ^{ns}	0.03 ^{ns}	0.65 ^{***}
*, **, *** and refer to significant level at 0.05, 0.01 and 0.001, ns; non-significant								
PH; plant height, DTH; day to heading, DTM; day to maturity, GNPS; grain number per spike, GYPS; grain yield per spike, TKW; thousand kernel weight, SL; spike length, NSPS; number of spikelets per spike								

Table 4

Correlation between traits and spike index under heat stress for two seasons (Above, bold season 1, below season 2).

Traits	PH	DTH	DTM	GNPS	GYPs	TKW	SL	NSPS	SI
PH	0.42 ^{***}	0.33^{***}	0.86^{***}	-0.21 [*]	-0.21 [*]	-0.16 ^{ns}	0.20 [*]	-0.16 ^{ns}	-0.21 [*]
DTH	0.48 ^{***}	0.89 ^{***}	0.77^{**}	-0.64^{**}	-0.61^{**}	-0.39^{**}	0.27^{**}	-0.46^{***}	-0.65^{***}
DTM	0.43 ^{***}	0.78 ^{***}	0.41 ^{***}	-0.49^{***}	-0.48^{***}	-0.33^{***}	0.28^{**}	-0.36^{***}	-0.49^{***}
GNPS	-0.10 ^{ns}	-0.48 ^{***}	-0.36 ^{***}	0.87 ^{***}	0.71^{***}	0.30^{**}	-0.21 [*]	0.67^{***}	0.92^{***}
GYPs	-0.09 ^{ns}	-0.49 ^{***}	-0.36 ^{***}	0.82 ^{***}	0.72 ^{***}	0.55^{***}	0.04 ^{ns}	0.56^{***}	0.82^{***}
TKW	-0.28 ^{**}	-0.53 ^{***}	-0.43 ^{***}	0.36 ^{***}	0.64 ^{***}	0.67 ^{***}	0.08^{ns}	0.34^{***}	0.61^{***}
SL	0.18 ^{ns}	0.23 [*]	0.15 ^{ns}	0.13 ^{ns}	0.01 ^{ns}	-0.09 ^{ns}	0.81 ^{***}	-0.13^{ns}	-0.02^{ns}
NSPS	0.29 ^{**}	-0.04 ^{ns}	0.09 ^{ns}	0.55 ^{***}	0.46 ^{***}	0.05 ^{ns}	0.13 ^{ns}	0.69 ^{***}	0.76^{***}
SI	0.13 ^{ns}	-0.74 ^{***}	-0.71 ^{***}	0.93 ^{***}	0.88 ^{***}	0.71 ^{***}	-0.06 ^{ns}	0.68 ^{***}	0.85 ^{***}
*, **, *** and refer to significant level at 0.05, 0.01 and 0.001, ns; non-significant									
PH; plant height, DTH; day to heading, DTM; day to maturity, GNPS; grain number per spike, GYPs; grain yield per spike, TKW; thousand kernel weight, SL; spike length, NSPS; number of spikelets per spike, SI; spike index including GNPS, GYPs, TKW, SL, and NSPS									

Selecting the best and lowest genotypes under heat stress.

As spike index showed a strong positive correlation with yield-related traits under heat stress in both growing seasons, selection of the genotypes was based on SI values, where a high value indicates high performance for these genotypes under heat stress. All genotypes were ranked from highest to lowest spike index values. The 20 highest as well as the lowest-performing genotypes under heat stress conditions were selected in each season separately. Selection results indicated that 13 high-yielding genotypes (Fig. 3b, Table 5) were consistently ranked among the top 20 in both seasons, whereas 11 genotypes consistently exhibited the lowest yield (Fig. 3c, Table 5). The thirteen high-yielding genotypes represented six genotypes from Egypt (three bread wheat genotypes and three durum wheat genotypes), two from Morocco, four from Oman, and one from Saudi Arabia. The eleven lowest-performing genotypes included three genotypes from Kenya, two from Germany, two from Kazakhstan, two from Iran, one from Afghanistan, and one from Canada. The performance of the two genotype sets, was compared for some measured traits under the four environments during the two seasons, as shown in (Supplementary figures S1 and S2).

Table 5
Selected genotypes under heat stress for two seasons.

Genotypes	Plant Name	Country
High	WAS_016	SAKHA94
	WAS_019	GIMMIZA10
	WAS_022	OMARA 004
	WAS_028	Beni Swief-7
	WAS_030	Sohag-3
	WAS_031	Sohag-5
	WAS_139	1004b
	WAS_140	1137
	WAS_150	Mufseggha
	WAS_153	Kule
	WAS_154	Musane
	WAS_157	Kule
	WAS_160	Baladi
Low	WAS_051	756
	WAS_080	LRS-1F193
	WAS_098	4453/31
	WAS_099	Janetzkje Somerwizen
	WAS_109	IWA8608569
	WAS_113	5401
	WAS_120	Pavlodarskaja I
	WAS_121	Meskren
	WAS_129	Rhodesian Sabanero
	WAS_131	Rhodesian Sabanero
	WAS_132	R64

Population structure and Genetic distance among the selected high-yielding genotypes.

The 103 genotypes that were evaluated were divided into subpopulations using the STRUCTURE analysis by Mourad et. al [16]. They were divided into three subpopulations, each containing 48, 46, and 9 genotypes, respectively. The mean average of all genotypes in spike index was calculated for each subpopulation separately under heat stress across the two seasons. The spike index results showed that the third subpopulation had the highest spike index compared to other subpopulations across two seasons, as shown in Fig. 4.

The genetic distance among the highly selected genotypes was calculated to determine the level of genetic diversity among these selected genotypes and distribution across three inferred subpopulations in Fig. 5. The dendrogram analysis identified two main groups. The genetic distance among the highly selected genotypes ranged from 0.236 to 0.627. The greatest genetic distance was observed between genotypes WAS_140 (**Morocco**) and WAS_160 (**Saudi Arabia**). Similarly, high genetic distances were detected between WAS_139 (**Morocco**) and WAS_153 (**Oman**), as well as between WAS_139 (**Morocco**) and

WAS_160 (**Saudi Arabia**), with all values approximately 0.62. In contrast, the lowest genetic distance was recorded between genotypes WAS_139 and WAS_140, both originating from Morocco. WAS_016 (**Egypt**) exhibited a high genetic distance from the other genotypes, ranging from 0.531 with genotype WAS_022 (**Egypt**) to 0.617 with genotype WAS_153 (**Oman**). The genotype WAS_157 (**Oman**) has a similar genetic distance with both genotypes WAS_160 (**Saudi Arabia**) and WAS_153 (**Oman**), as shown in Supplementary Table S4.

Discussion

The analysis of variance (ANOVA) for all traits under different environmental conditions.

Combined analysis of variance (ANOVA) revealed a high genetic variation among the genotypes for all measured traits; this diversity likely reflects that the genotypes originated from 14 countries. The combined ANOVA also showed significant differences between the two treatments for most traits, except plant height (PH) and number of spikelets per spike (NSPS), indicating that genotypes differed in their responses to high temperature. Non-significant differences between normal and heat stress for PH and NSPS were also reported by Sarkar et al [19]. Genotype-by-treatment interactions were significant for plant height (PH) and number of spikelets per spike (NSPS). The differences among genotypes (G) were highly significant, indicating considerable genetic variation among genotypes and high-yielding genotypes under heat stress can be distinguished and selected for future breeding programs [20, 21]. The genotype $\times$ year interaction was highly significant, indicating that genotype performance changed from one year to another. Genotype $\times$ treatment interaction was also highly significant, indicating that the genotypes react differently to normal or heat stress conditions, similar to results reported by Modarresi et. al [22]. The broad-sense heritability ranged from moderate to high for all measured traits, suggesting that the majority of variation among genotypes were due to genetic differences and hence selection for improving yield traits under heat stress is feasible [23].

The population consisted of highly diverse wheat genotypes from 14 countries, and high genetic variation was expected for all traits. This same population has previously been used to identify genes and markers associated with fungal disease resistance [24–27], heavy metal tolerance [28–30], salinity tolerance [31], and seedling-stage drought tolerance [32].

Effect of heat stress on the measured traits.

Heat stress highly affected all measured traits, and the reduction in traits resulting from heat stress was greater in the first season than in the second season due to the higher temperature in the first season compared to the second season. This indicates that genotypes were exposed to higher temperature waves in the second sowing date, in both growing seasons, at the heading, anthesis, and grain-filling stages, than those planted in the optimum sowing date at the respective growing stage. Late sowing dates have previously been used to expose wheat and barley at the heading, anthesis, and grain-filling stages to heat stress [10].

Grain yield per spike (GYPS) was the trait most affected by heat stress in both growing seasons, which is consistent with the findings of Modarresi et. al [22], who reported that high temperature significantly decreased all traits, with grain yield showing the greatest reduction (46.63%). Heat stress has a detrimental effect on wheat at every stage of development, but it is most detrimental to grain yield and quality during the reproductive stage [33]. Pollen sterility is induced when temperatures exceed 31°C in the period shortly before anthesis. This prevents pollen from fertilizing ovules and producing viable seeds, causing pre-anthesis disruptions that negatively affect yield and its components [34]. In our study, the genotypes were exposed to ~ 32–38°C and 31 and 37°C in the two growing seasons, respectively, which is higher than reported in earlier studies. Pollen abortion is the primary cause of yield and yield-related trait decrease under stress [35], demonstrating that heat stress decreased pollen viability during the reproductive stage [36]. High temperatures around anthesis (> 30°C) reduce pollen fertility and can cause grain sterility. Therefore, heat stress decreases grain number per spike, shortens grain-filling duration, and reduces grain yield through pollen sterility [37]. Moreover, the number of endosperm cells in developing grains is reduced by heat stress, leading to smaller grain size and lower thousand-kernel weight (TKW), and consequently reduced grain quality [38]. In the current study, spike length was the trait least affected by heat stress. Spike length is a more structurally

determined trait than grain number, grain weight, or final yield, all of which are directly impaired during anthesis and grain filling, it tends to be less affected by heat stress[22]. Exposure to high temperature during early booting significantly reduced days to heading and days to maturity in wheat, indicating accelerated phenological development under heat stress [39].

The phenotypic correlations among all traits under normal and heat stress conditions in two seasons.

In all four environments, there were high correlations between GYPS and GNPS, NSPS, and TKW. High trait correlations may allow indirect selection to improve several traits at once. Grain yield was significantly and positively correlated with thousand-kernel weight and number of grains per spike, indicating that higher grain yield per spike is possible in genotypes that show high thousand-kernel weight, number of spikelets per spike, and grains number per spike. These results are consistent with Shehrawat et. al [40]. GYPS was negatively correlated with DTH and DTM, indicating that early-heading genotypes tend to have higher grain yield per spike than late-heading genotypes[41] Early heading allowed genotypes to avoid heat stress and improve grain yield. A negative correlation was found between grain yield per plant and plant height under both normal and heat stress conditions [42]. Reducing plant height increased wheat grain yield, reflected by a significant negative association between grain yield (GY) and plant height (PH) in both environments [43]. The positive correlations of grain yield per spike with both grain number per spike, spikelets per spike, and thousand-kernel weight together with the positive associations among spike traits under heat stress in both seasons, suggest that selecting for grain yield per spike will simultaneously improve these related spike traits.

Comparing the correlation patterns among traits under normal and heat-stress conditions revealed that heat stress reorganized trait associations, making yield more dependent on grain number, kernel weight, and stress-adaptive traits, while several correlations observed under normal conditions weakened or changed under stress. These correlations suggest that heat stress did not simply weaken performance uniformly, but instead shifted trait interdependence toward a more selective reproductive survival network centered on spike fertility, grain set, and grain filling.

Selection index was used to include GNPS, GYPS, TKW, SL, and NSPS under heat stress in both growing seasons. The spike index (SI) had high significant correlation with all yield-related traits and the correlation among the spike indices between the two years was also highly significant, indicating that breeders can rely on spike index for selecting stable and high-yielding genotypes under heat stress [40]. The stronger negative association of heading and maturity time with spike index and yield-related traits under heat stress further indicates that earliness was adaptive in this population, most likely through escape from high temperature during sensitive reproductive stages. That interpretation is consistent with field and multi-environment studies showing that yield under heat is often negatively associated with heading or maturity time, and with mechanistic evidence that grain number is especially vulnerable to heat in the narrow pre-flowering window, whereas grain weight is additionally penalized by post-flowering heat. Notably, spike length remained weakly associated with spike index and most yield traits in both environments, suggesting that larger spike structure alone did not ensure heat resilience. This agrees with reports that direct partitioning into grain, harvest index, and spike fertility are better indicators of heat adaptation than gross morphological traits. It also supports prioritizing grain number per spike, grain weight per spike, thousand-kernel weight, and fertility-related indices over spike length as practical selection criteria

The multi-trait selection index is a quantitative method that enables the simultaneous selection of several traits by assigning weights to each trait according to its relative breeding or economic importance. Breeders rarely focus on a single trait and disregard all others during selection[39]. Selection should be carried out on several traits at the same time[44] This strategy helps breeders maximize overall genetic gain by focusing on the combined value of all target traits, instead of improving each trait separately. This method was previously used for improving drought tolerance in wheat at the seedling stage[32] and spike productivity at the adult growth stage [23] In faba bean, a selection index was used to select high frost-tolerant genotypes at the seedling stage under controlled conditions [45], high-yielding genotypes under winter hardiness conditions [46]and genotypes with superior seed traits[47]

Selecting the best genotypes under heat stress.

Due to high correlation of SI between the two-growing season under heat stress, it was used to select the highest high-yielding genotypes. Selection should be applied to multiple traits simultaneously, enabling breeders to maximize overall genetic gain by targeting the combined value of all key traits rather than improving each one in isolation. Thirteen genotypes from four countries (Egypt, Morocco, Oman, and Saudi Arabia).

showed high SI under heat stress in both seasons. Out of thirteen selected genotypes, Beni Swief-7 was reported as a heat-tolerant genotype [48]. Sohag-5 genotype showed the highest grain yield under conditions of water and heat stress [49], and Sohag-3 was reported as a drought-tolerant genotype at the seedling stage [50]. In addition, 11 genotypes from six countries (Afghanistan, Canada, Germany, Iran, Kazakhstan, and Kenya) were identified as the lowest-performing across both growing seasons, making them valuable candidates for use as heat-susceptible checks in subsequent heat tolerance screening trials. Moreover, the susceptible genotypes can be crossed with tolerant genotypes to develop bi-parental populations to identify major QTLs associated with heat tolerance in wheat. Susceptible genotypes can be used for gene validation studies to confirm the expression of genes associated with heat tolerance in wheat

Population structure and genetic diversity for improving heat tolerance.

Studying population structure is highly useful for understanding the genetic diversity of the tested genotypes and represents an essential first step in association mapping studies. Population structure analysis divided the 103 genotypes into three subpopulations (SP1: 48, SP2: 46, and SP3: 9) [16]. The SP3 showed the highest mean spike index under heat stress across both seasons. This suggests that SP3 may carry unique alleles or favorable haplotypes associated with heat tolerance, despite its small size. These results are consistent with previous studies demonstrating that population structure analysis can effectively identify genetically distinct groups with contrasting stress responses in wheat and other cereals[51, 52]Integrating subpopulation membership with phenotypic selection can help breeders maximize genetic gain while maintaining sufficient genetic diversity in breeding populations. Crossing high-performing genotypes from SP3 with genotypes from SP1 or SP2 can generate transgressive segregants through the combination of complementary alleles, thereby accelerating the development of heat-tolerant cultivars. This strategy is particularly effective when parental selection is based on both phenotypic performance (spike index under heat stress) and genetic distance (subpopulation membership), as it reduces the risk of crossing closely related genotypes that may share similar susceptibility alleles. The integration of population structure and phenotypic data was also used for selecting highly-divergent drought tolerant genotypes in rice [53]. Furthermore, the identification of subpopulations with contrasting heat responses facilitates marker-trait association studies and the discovery of candidate genes underlying heat tolerance. Genotypes from SP3 represent promising donors for marker-assisted selection programs aimed at introgressing heat tolerance alleles into elite breeding lines, ultimately enhancing the resilience of wheat cultivars to rising temperatures.

Genetic diversity among these genotypes was expected because the genotypes were collected from more than one geographical region. Analyzing the genetic diversity among these genotypes is very important in selecting high-performing genotypes under heat stress conditions. The genetic distance analysis among highly selected genotypes revealed a wide range of diversity (0.236–0.627), indicating substantial genetic distance (diversity) within this subset. The greatest genetic distance was observed between WAS_140 (Morocco) and WAS_160 (Saudi Arabia), WAS_139 (Morocco) and WAS_153 (Oman) and WAS_160 (Saudi Arabia), all with values approximately 0.627. In contrast, the lowest genetic distance was recorded between WAS_139 and WAS_140, both originating from Morocco, reflecting their close genetic relationship and shared geographic origin. Notably, WAS_016 (Egypt) had a high genetic distance with all selected genotypes. Selecting parental combinations based on both phenotypic performance and genetic distance can maximize heterosis and generate transgressive segregants with enhanced heat tolerance. Specifically, crossing distantly related genotypes. For example, WAS_140 (Morocco, SP1) and WAS_160 (Saudi Arabia, SP2) can be very useful for crossing because they are highly divergent (0.62) and also belong to different subpopulations. This will help in exploiting complementary alleles (associated with heat tolerance) from diverse geographic origins. Crossing closely related genotypes, on the other hand, such as WAS_139 and WAS_140 (both from Morocco) may not be useful for producing wheat cultivars with high agronomic traits compared to WAS_140 and WAS_160. In addition, crossing WAS_016 (Egypt) with highly divergent exotic genotypes, particularly WAS_153

(Oman, SP2), WAS_157 (Oman, SP2), and WAS_160, may help combine heat-tolerance alleles while retaining adaptation to Egyptian environments. This rationale is consistent with evidence that favorable allele combinations from diverse germplasm can improve grain yield under heat stress, and that exotic or wild alleles can be incorporated into breeding programs to strengthen climate resilience

The distribution of highly selected genotypes across the three inferred subpopulations further supports the integration of population structure information into breeding strategies, enabling breeders to maintain genetic diversity while targeting heat tolerance. Genetic diversity among genotypes is highly beneficial in breeding programs for improving target traits by selecting the best-performing and highly divergent genotypes[54, 55]

Conclusion

Heat stress significantly affected spike-related traits and their associated characteristics, with grain yield per spike being the most strongly reduced trait under stress conditions. Based on integrated phenotypic and genotypic variation, 13 heat-tolerant and 11 heat-sensitive genotypes were identified. Among the tolerant genotypes, WAS_016 and WAS_139 showed the smallest reductions in grain yield per spike. Conversely, WAS_113 and WAS_080 exhibited the greatest reductions in grain yield per spike under delayed sowing conditions, highlighting their high susceptibility to terminal heat stress. The combined use of population structure, genetic diversity, and phenotypic data proved effective for identifying promising parental lines for wheat improvement under heat stress. Notably, WAS_140 (Morocco) and WAS_160 (Saudi Arabia) exhibited the greatest genetic distance among the selected genotypes, suggesting that their hybridization could broaden genetic variability and support the development of superior heat-tolerant wheat progeny.

Declarations

Author contributions:

HAS: Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. AAS: Investigation, Supervision. ARMR: Investigation, Supervision. AS: Conceptualization, Methodology, Supervision, Writing – review & editing.

Funding

This work was supported by the Science, Technology & Innovation Funding Authority (STDF), Egypt.

Data Availability

All data of this article are in the supplementary files

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Clinical Trial Number

Not applicable.

Competing interests

The authors declare no conflicts of interest.

References

1. Ghazy AI, Al Ateeq TK, Ibrahim EI, Abdel-Haleem H, Attia KA, Azab O, et al. Selection of heat stress tolerant wheat genotypes for desert environments. *Sci Rep.* 2025;15. <https://doi.org/10.1038/s41598-025-20450-7>.
2. FAOSTAT. <http://www.fao.org/faostat/en/#data/QCL>. 2024.
3. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: An overview. *Environ Exp Bot.* 2007;61:199–223. <https://doi.org/10.1016/j.envexpbot.2007.05.011>.
4. Rehman HU, Tariq A, Ashraf I, Ahmed M, Muscolo A, Basra SMA, et al. Evaluation of physiological and morphological traits for improving spring wheat adaptation to terminal heat stress. *Plants.* 2021;10:1–15. <https://doi.org/10.3390/plants10030455>.
5. Ullah A, Nadeem F, Nawaz A, Siddique KHM, Farooq M. Heat stress effects on the reproductive physiology and yield of wheat. *J Agron Crop Sci.* 2022;208:1–17. <https://doi.org/10.1111/jac.12572>.
6. Poudel PB, PMR. Heat Stress Effect and Tolerance in Wheat: A Review. *J Bio Today's World, &.* 2020; 9(4):217. 2020.
7. Ibrahim K, Said A. Grain Yield Stability of New Bread Wheat Genotypes (*Triticum aestivum* L.) under Normal and Heat Stress Conditions. *Egypt J Agron.* 2020;0:0–0. <https://doi.org/10.21608/agro.2020.32118.1216>.
8. Khairnar SS, Bagwan JH, Yashavantha Kumar KJ, Baviskar VS, Honrao BK, Surve VD, et al. Studies on genetic variability parameters and character association in bread wheat (*Triticum aestivum* L.) under timely and late sown environments of irrigated condition. *Electron J Plant Breed.* 2018;9:190–8. <https://doi.org/10.5958/0975-928X.2018.00023.6>.
9. Hamam KA, Khaled AGA, Zakaria MM, GENETIC STABILITY AND, DIVERSITY IN. YIELD COMPONENTS OF SOME WHEAT GENOTYPES THROUGH SEASONS AND HEAT STRESS UNDER DIFFERENT LOCATIONS. 2015.
10. Sallam A, Amro A, EL-Akhdar A, Dawood MFA, Kumamaru T, Stephen Baenziger P. Genetic diversity and genetic variation in morpho-physiological traits to improve heat tolerance in Spring barley. *Mol Biol Rep.* 2018;45:2441–53. <https://doi.org/10.1007/s11033-018-4410-6>.
11. Utz FH. PLABSTAT: A computer program for statistical analysis of plant breeding experiments. 1997.
12. R core Team. A language and environment for statistical computing. 2025.
13. Olivoto T, Lúcio ADC. metan: An R package for multi-environment trial analysis. *Methods Ecol Evol.* 2020;11:783–9. <https://doi.org/10.1111/2041-210X.13384>.
14. Hazel LN, THE GENETIC BASIS FOR CONSTRUCTING SELECTION I. N D E X E S. 1943.
15. SMITH HF, A DISCRIMINANT FUNCTION FOR, PLANT SELECTION. *Ann Eugen.* 1936;7:240–50. <https://doi.org/10.1111/j.1469-1809.1936.tb02143.x>.
16. Mourad AMI, Belamkar V, Baenziger PS. Molecular genetic analysis of spring wheat core collection using genetic diversity, population structure, and linkage disequilibrium. *BMC Genomics.* 2020;21:434. <https://doi.org/10.1186/s12864-020-06835-0>.
17. Dray S, Dufour A-B. *Journal of Statistical Software The ade4 Package: Implementing the Duality Diagram for Ecologists.* 2007.
18. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics.* 2007;23:2633–5. <https://doi.org/10.1093/bioinformatics/btm308>.
19. Sarkar S, Barma NCD, Ahmed JU, Waza SA, Islam AKMA. Morpho-physiological characterization for identification of heat tolerant wheat genotypes. *Discover Agric.* 2025;3. <https://doi.org/10.1007/s44279-025-00223-6>.
20. Rezaei V, Majidi MM, Mirlohi A, Saeidi G. Productivity and quality-related traits of wheat germplasm affected by heat stress. *PLoS ONE.* 2026;21. <https://doi.org/10.1371/journal.pone.0333505>. 4 April.
21. Devi K, Chahal S, Venkatesh K, Singh S, Tripathi SC, Mamrutha HM, et al. Identification of Wheat Genotypes Resilient to Terminal Heat Stress Using GGE Biplot Analysis. *J Soil Sci Plant Nutr.* 2022;22:3386–98. <https://doi.org/10.1007/s42729-022-00894-w>.
22. Modarresi M, Mohammadi V, Zali A, Mardi M. Response of wheat yield and yield related traits to high temperature. *Cereal Res Commun.* 2010;38:23–31. <https://doi.org/10.1556/CRC.38.2010.1.3>.

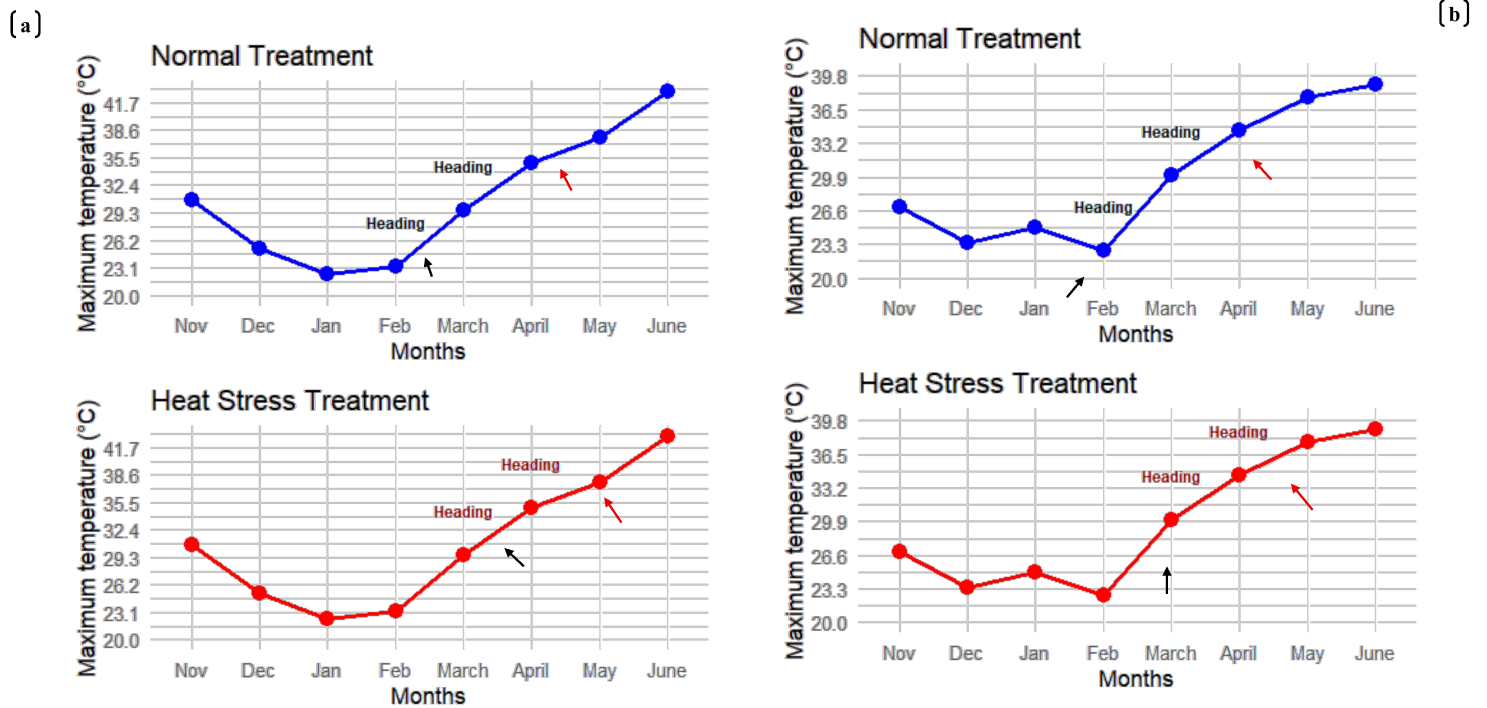
23. Sallam A, Hashem M, Ahmed AAM, Ismail SM, Baenziger PS, Börner A. Genetic and Phenotypic Associations between Peduncle Characteristics and Spike Productivity in Wheat Under Drought and Normal Conditions. 2025. <https://doi.org/10.21203/rs.3.rs-6983003/v1>
24. Esmail SM, Omar GE, Mourad AMI. In-Depth Understanding of the Genetic Control of Stripe Rust Resistance (*Puccinia striiformis* f. sp. *tritici*) Induced in Wheat (*Triticum aestivum*) by *Trichoderma asperellum* T34. *Plant Dis.* 2023;107:457–72. <https://doi.org/10.1094/PDIS-07-22-1593-RE>.
25. Mourad AMI, Hamdy RM, Esmail SM. Novel genomic regions on chromosome 5B controlling wheat powdery mildew seedling resistance under Egyptian conditions. *Front Plant Sci.* 2023;14. <https://doi.org/10.3389/fpls.2023.1160657>.
26. Mourad AMI, Abou-Zeid MA, Eltaher S, Baenziger PS, Börner A. Identification of candidate genes and genomic regions associated with adult plant resistance to stripe rust in spring wheat. *Agronomy.* 2021;11. <https://doi.org/10.3390/agronomy11122585>.
27. Abou-Zeid MA, Mourad AMI. Genomic regions associated with stripe rust resistance against the Egyptian race revealed by genome-wide association study. *BMC Plant Biol.* 2021;21. <https://doi.org/10.1186/s12870-020-02813-6>.
28. Mourad AMI, Sallam A, Farghaly KA, Börner A. Detailed genetic analyses highlight genetic variation and genomic regions for lead tolerance in spring wheat. *Front Agron.* 2025;7. <https://doi.org/10.3389/fagro.2025.1428366>
29. Mourad AMI, Baghdady S, Abdel-Aleem FAZM, Jazeri RM, Börner A. Novel Genomic Regions and Gene Models Controlling Copper and Cadmium Stress Tolerance in Wheat Seedlings. *Agronomy.* 2024;14. <https://doi.org/10.3390/agronomy14122876>.
30. Sharshar AM, Esmail SM, Elmoghazy DE, Elhag WA, Genedy MS, Mourad AMI. Effect of Combined Alkaline-saline and Stripe Rust Stresses on Physiological and Yield Traits in Bread Wheat. *J Crop Health.* 2025;77. <https://doi.org/10.1007/s10343-024-01067-8>.
31. Hasseb NM, Sallam A, Karam MA, Gao L, Wang RRC, Moursi YS. High-LD SNP markers exhibiting pleiotropic effects on salt tolerance at germination and seedlings stages in spring wheat. *Plant Mol Biol.* 2022;108:585–603. <https://doi.org/10.1007/s11103-022-01248-x>.
32. Sallam A, Dawood MFA, Jarquín D, Mohamed EA, Hussein MY, Börner A, et al. Genome-wide scanning to identify and validate single nucleotide polymorphism markers associated with drought tolerance in spring wheat seedlings. *Plant Genome.* 2024;17. <https://doi.org/10.1002/tpg2.20444>.
33. Fan Y, Ma C, Huang Z, Abid M, Jiang S, Dai T, et al. Heat priming during early reproductive stages enhances thermo-tolerance to post-anthesis heat stress via improving photosynthesis and plant productivity in winter wheat (*Triticum aestivum* L). *Front Plant Sci.* 2018;9. <https://doi.org/10.3389/fpls.2018.00805>.
34. Roychowdhury R, Zilberman O, Chandrasekhar K, Curzon A, Nashef K, Abbo S et al. Pre-anthesis spike growth dynamics and its association to yield components among elite bread wheat cultivars (*Triticum aestivum* L. spp.) under Mediterranean climate. 2023. <https://doi.org/10.1101/2023.01.08.523132>
35. Ji X, Shiran B, Wan J, Lewis DC, Jenkins CLD, Condon AG, et al. Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. *Plant Cell Environ.* 2010;33:926–42. <https://doi.org/10.1111/j.1365-3040.2010.02130.x>.
36. Khan I, Wu J, Sajjad M. Pollen viability-based heat susceptibility index (HSI_{pv}): A useful selection criterion for heat-tolerant genotypes in wheat. *Front Plant Sci.* 2022;13. <https://doi.org/10.3389/fpls.2022.1064569>.
37. Sihag P, Kumar U, Sagwal V, Kapoor P, Singh Y, Mehla S, et al. Effect of terminal heat stress on osmolyte accumulation and gene expression during grain filling in bread wheat (*Triticum aestivum* L). *Plant Genome.* 2024;17. <https://doi.org/10.1002/tpg2.20307>.
38. Girousse C. Heat shock exposure during early wheat grain development can reduce maximum endosperm cell number but not necessarily final grain dry mass. *PLoS ONE.* 2023;18. <https://doi.org/10.1371/journal.pone.0285218>. 4 APRIL.
39. Xu J, Lowe C, Hernandez-Leon SG, Dreisigacker S, Reynolds MP, Valenzuela-Soto EM et al. The effects of brief heat during early booting on reproductive, developmental and physiological performance in common wheat (*Triticum aestivum* L.).

2022. <https://doi.org/10.1101/2022.02.20.481180>

40. Shehrawat S, Kumar Y. Genetic Architecture of Morpho-Physiological Traits in Wheat Accessions under Terminal Heat Stress. 2021.
41. Bhandari R, Panthi B, Nyaupane S, Shrestha S, Sharma P, Gupta RK, et al. Phenotypic Correlation, Path Analysis, and Quantitative Trait-Based Selection of Elite Wheat Genotypes Under Heat Stress Conditions in The Terai Region of Nepal. *Makara J Sci.* 2024;28:216–25. <https://doi.org/10.7454/mss.v28i3.2209>.
42. Riaz MW, Yang L, Yousaf MI, Sami A, Mei XD, Shah L, et al. Effects of heat stress on growth, physiology of plants, yield and grain quality of different spring wheat (*Triticum aestivum* L.) genotypes. *Sustain (Switzerland).* 2021;13:1–18. <https://doi.org/10.3390/su13052972>.
43. Grover G, Sharma A, Gill HS, Srivastava P, Bains NS. Rht8 gene as an alternate dwarfing gene in elite Indian spring wheat cultivars. *PLoS ONE.* 2018;13. <https://doi.org/10.1371/journal.pone.0199330>.
44. Al-Khayri JM, Jain SM, Johnson DV. *Advances in plant breeding strategies: Cereals.* Springer International Publishing; 2019. <https://doi.org/10.1007/978-3-030-23108-8>.
45. Sallam A, Martsch R, Moursi YS. Genetic variation in morpho-physiological traits associated with frost tolerance in faba bean (*Vicia faba* L.). *Euphytica.* 2015;205:395–408. <https://doi.org/10.1007/s10681-015-1395-2>.
46. Sallam A, Ghanbari M, Martsch R. Genetic analysis of winter hardiness and effect of sowing date on yield traits in winter faba bean. *Sci Hortic.* 2017;224:296–301. <https://doi.org/10.1016/j.scienta.2017.04.015>.
47. Sallam A, Mahmoud SM, Amro A, Elfarash A, Börner A. Effects of genotype × environment interactions on the morphological and genetic aspects of the seed characteristics of faba beans. *Plant Genome.* 2025;18. <https://doi.org/10.1002/tpg2.70133>.
48. Gab Alla MMM, Hussein EMA. Screening the yield potential of some bread and durum wheat under new valley conditions using some statistical procedures. *Egypt J Agricultural Res.* 2025;103:135–55. <https://doi.org/10.21608/ejar.2025.361179.1644>.
49. Eissa ST, Omnya, Elmoselhy MA, Arab SA. Journal of Plant Production Journal homepage & Evaluation of some Durum Wheat Genotypes under Different Stress Conditions. *J Plant Prod Mansoura Univ.* 2022;13:597–604. <https://doi.org/10.21608/jpp.2022.147865.1139>.
50. Ahmed AAM, Dawood MFA, Elfarash A, Mohamed EA, Hussein MY, Börner A, et al. Genetic and morpho-physiological analyses of the tolerance and recovery mechanisms in seedling stage spring wheat under drought stress. *Front Genet.* 2022;13. <https://doi.org/10.3389/fgene.2022.1010272>.
51. Eltaher S, Sallam A, Belamkar V, Emara HA, Nower AA, Salem KFM, et al. Genetic diversity and population structure of F3:6 Nebraska Winter wheat genotypes using genotyping-by-sequencing. *Front Genet.* 2018. <https://doi.org/10.3389/fgene.2018.00076>. 9 MAR.
52. Eltaher S, Mourad AMI, Baenziger PS, Wegulo S, Belamkar V, Sallam A. Identification and Validation of High LD Hotspot Genomic Regions Harboring Stem Rust Resistant Genes on 1B, 2A (Sr38), and 7B Chromosomes in Wheat. *Front Genet.* 2021;12. <https://doi.org/10.3389/fgene.2021.749675>.
53. Ghazy MI, Salem KFM, Sallam A. Utilization of genetic diversity and marker-trait to improve drought tolerance in rice (*Oryza sativa* L.). *Mol Biol Rep.* 2021;48:157–70. <https://doi.org/10.1007/s11033-020-06029-7>.
54. Babu BK, Meena V, Agarwal V, Agrawal PK. Population structure and genetic diversity analysis of Indian and exotic rice (*Oryza sativa* L.) accessions using SSR markers. *Mol Biol Rep.* 2014;41:4329–39. <https://doi.org/10.1007/s11033-014-3304-5>.
55. Salem KFM, Sallam A. Analysis of population structure and genetic diversity of Egyptian and exotic rice (*Oryza sativa* L.) genotypes. *C R Biol.* 2016;339:1–9. <https://doi.org/10.1016/j.crvi.2015.11.003>.

Figures

Figure 1: The maximum temperatures and heading periods in season 1 (a) and season 2 (b).

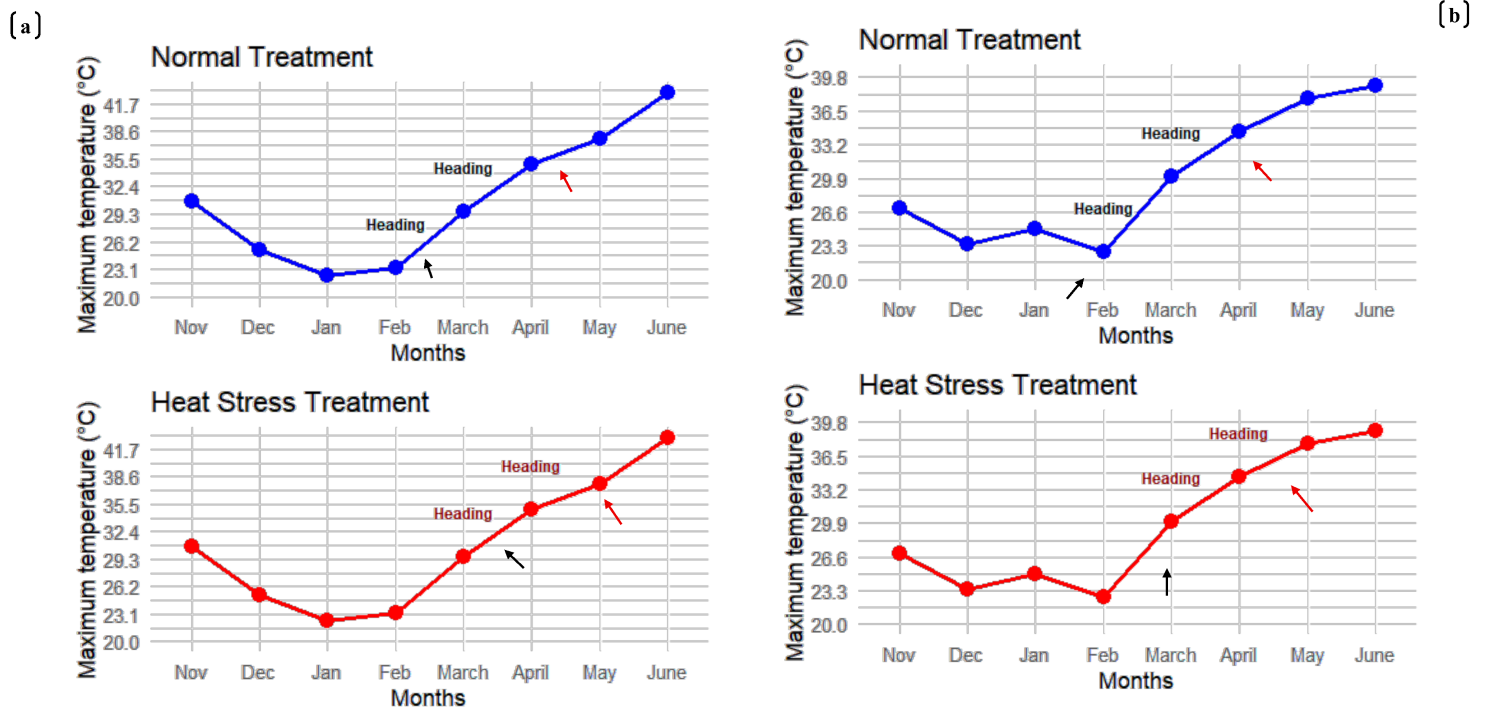


The black arrow indicates the date of heading for the first genotype, while the red arrow indicates the date of heading for the last genotype across the four environments.

Figure 1

See image above for figure legend

Figure 1: The maximum temperatures and heading periods in season 1 (a) and season 2 (b).

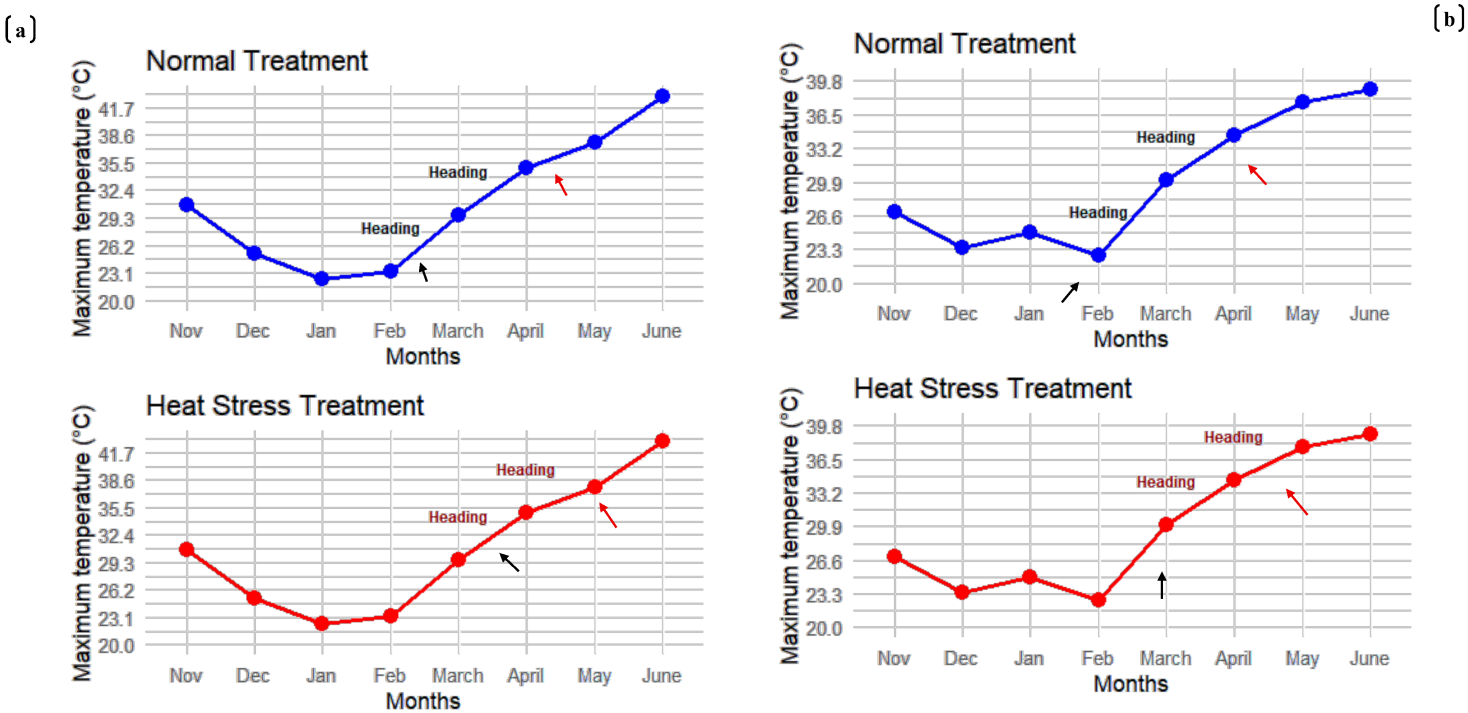


The black arrow indicates the date of heading for the first genotype, while the red arrow indicates the date of heading for the last genotype across the four environments.

Figure 1

See image above for figure legend

Figure 1: The maximum temperatures and heading periods in season 1 (a) and season 2 (b).



(b)

Normal Treatment

Month	Temperature (°C)
Nov	26.6
Dec	23.3
Jan	26.6
Feb	23.3
March	29.9
April	33.2
May	36.5
June	39.8

Heat Stress Treatment

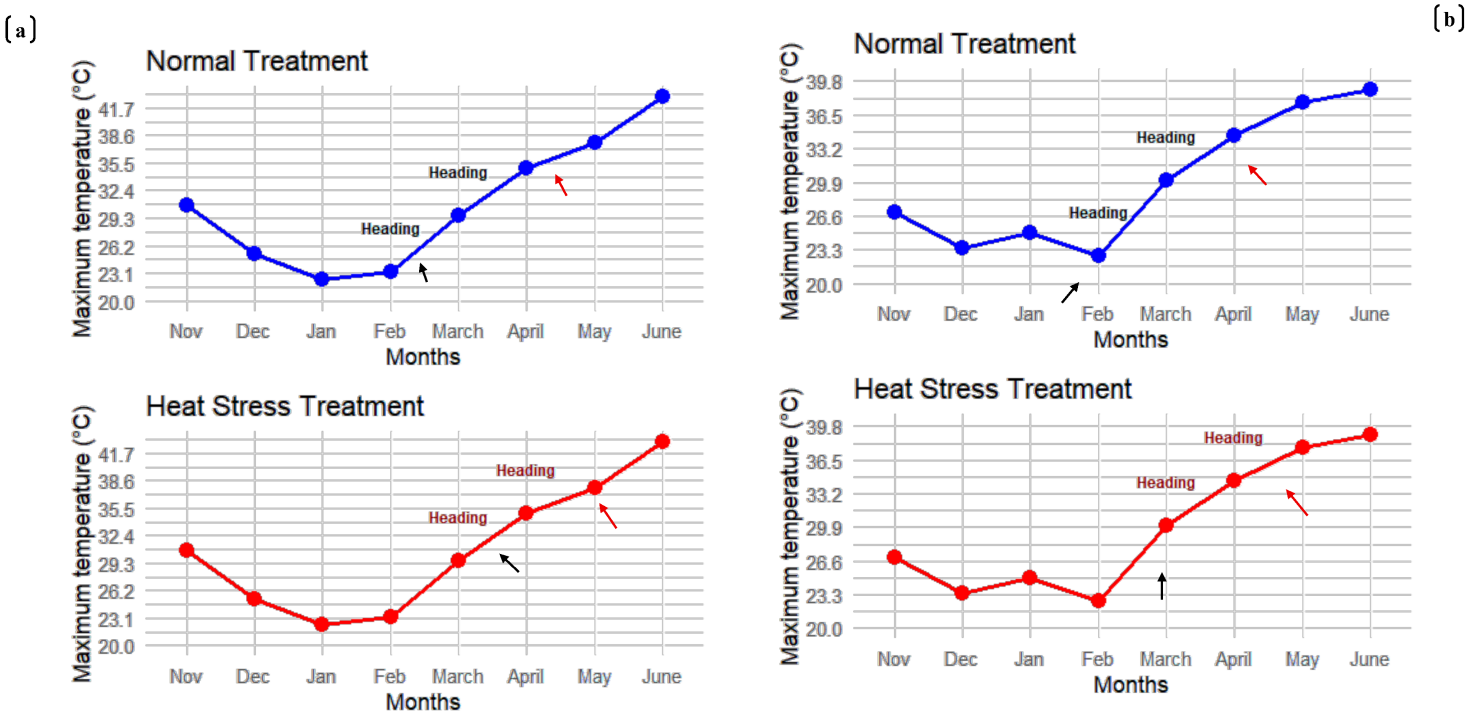
Month	Temperature (°C)
Nov	26.6
Dec	23.3
Jan	26.6
Feb	23.3
March	29.9
April	33.2
May	36.5
June	39.8

The black arrow indicates the date of heading for the first genotype, while the red arrow indicates the date of heading for the last genotype across the four environments.

Figure 1

See image above for figure legend

Figure 1: The maximum temperatures and heading periods in season 1 (a) and season 2 (b).



(b)

Normal Treatment

Month	Temperature (°C)
Nov	26.6
Dec	23.3
Jan	26.6
Feb	23.3
March	29.9
April	33.2
May	36.5
June	39.8

Heat Stress Treatment

Month	Temperature (°C)
Nov	26.6
Dec	23.3
Jan	26.6
Feb	23.3
March	29.9
April	33.2
May	36.5
June	39.8

The black arrow indicates the date of heading for the first genotype, while the red arrow indicates the date of heading for the last genotype across the four environments.

Figure 1

See image above for figure legend

Figure 2: Reduction of traits due to heat stress for two seasons.

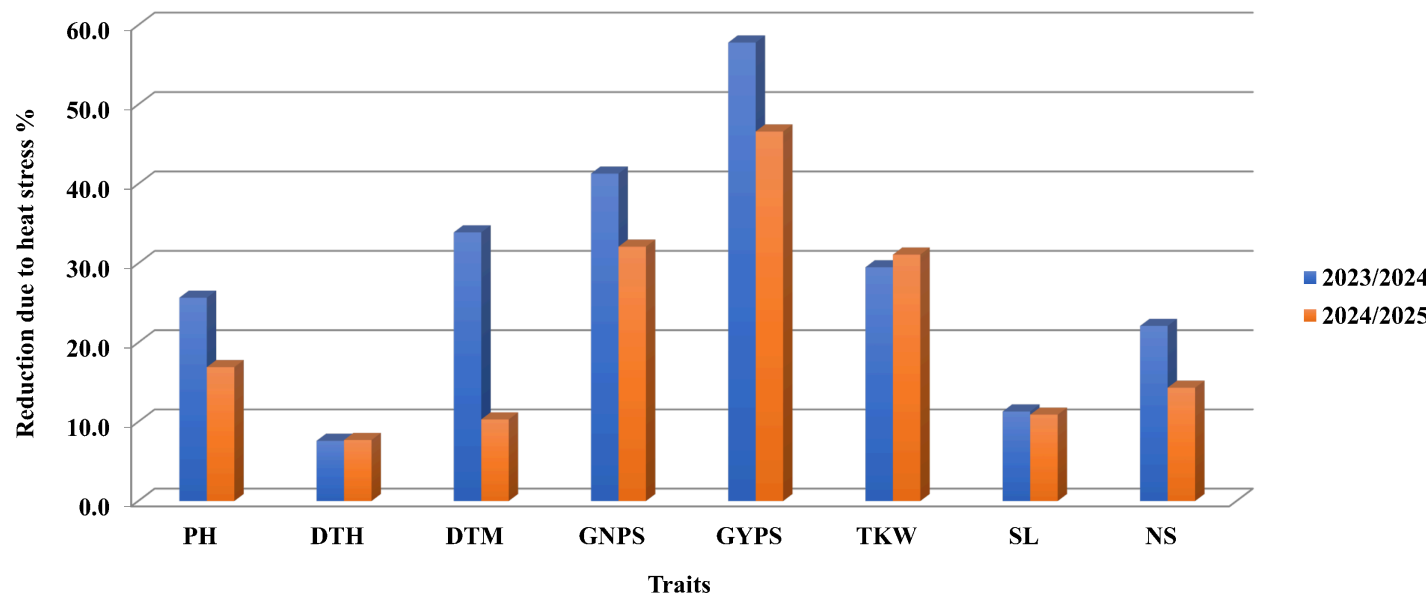


Figure 2

See image above for figure legend

Figure 2: Reduction of traits due to heat stress for two seasons.

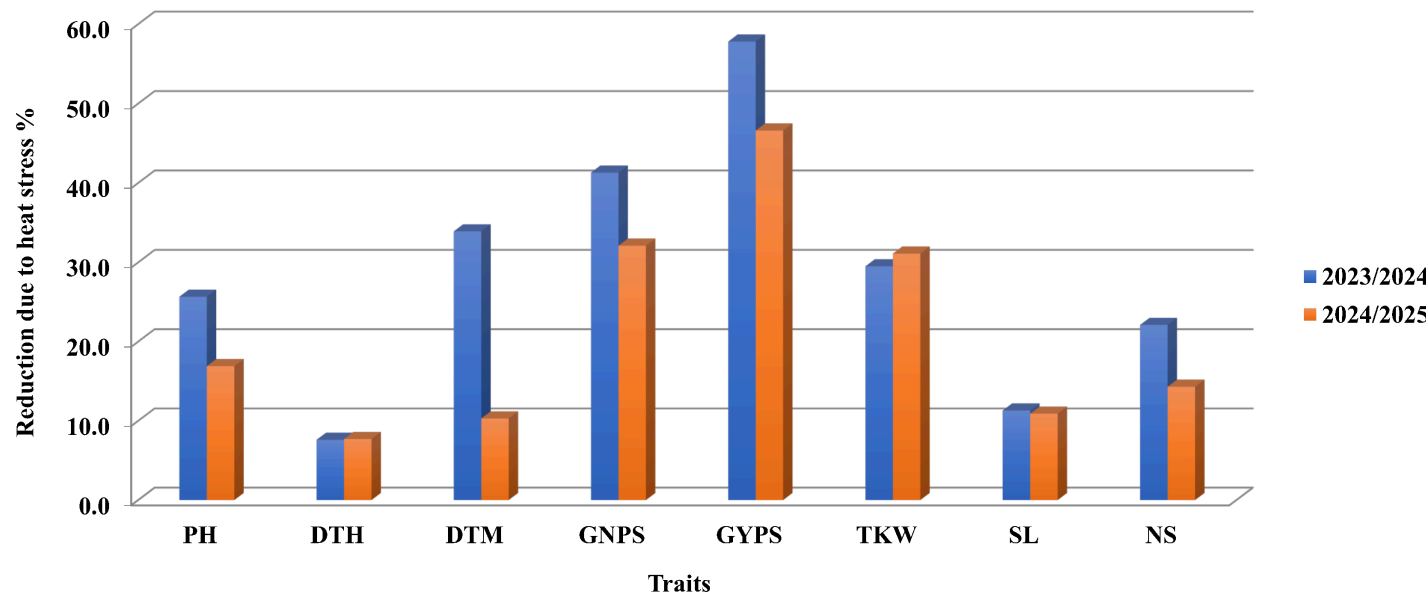


Figure 2

See image above for figure legend

Figure 2: Reduction of traits due to heat stress for two seasons.

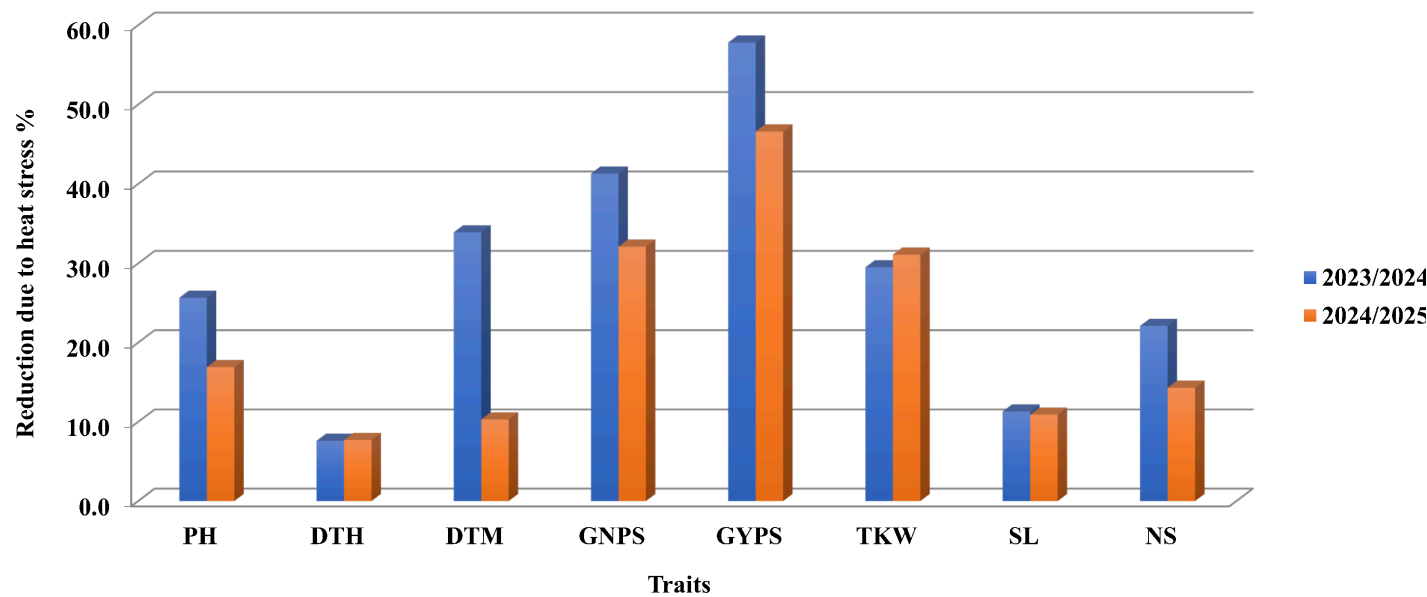


Figure 2

See image above for figure legend

Figure 2: Reduction of traits due to heat stress for two seasons.

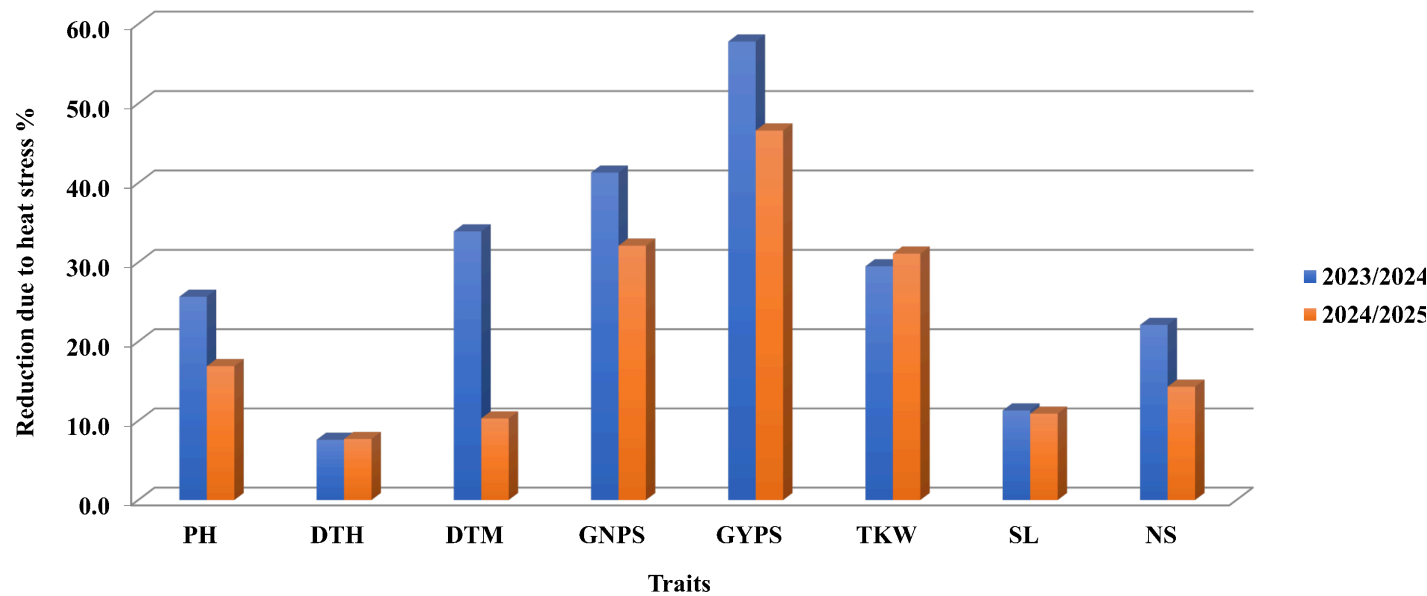


Figure 2

See image above for figure legend

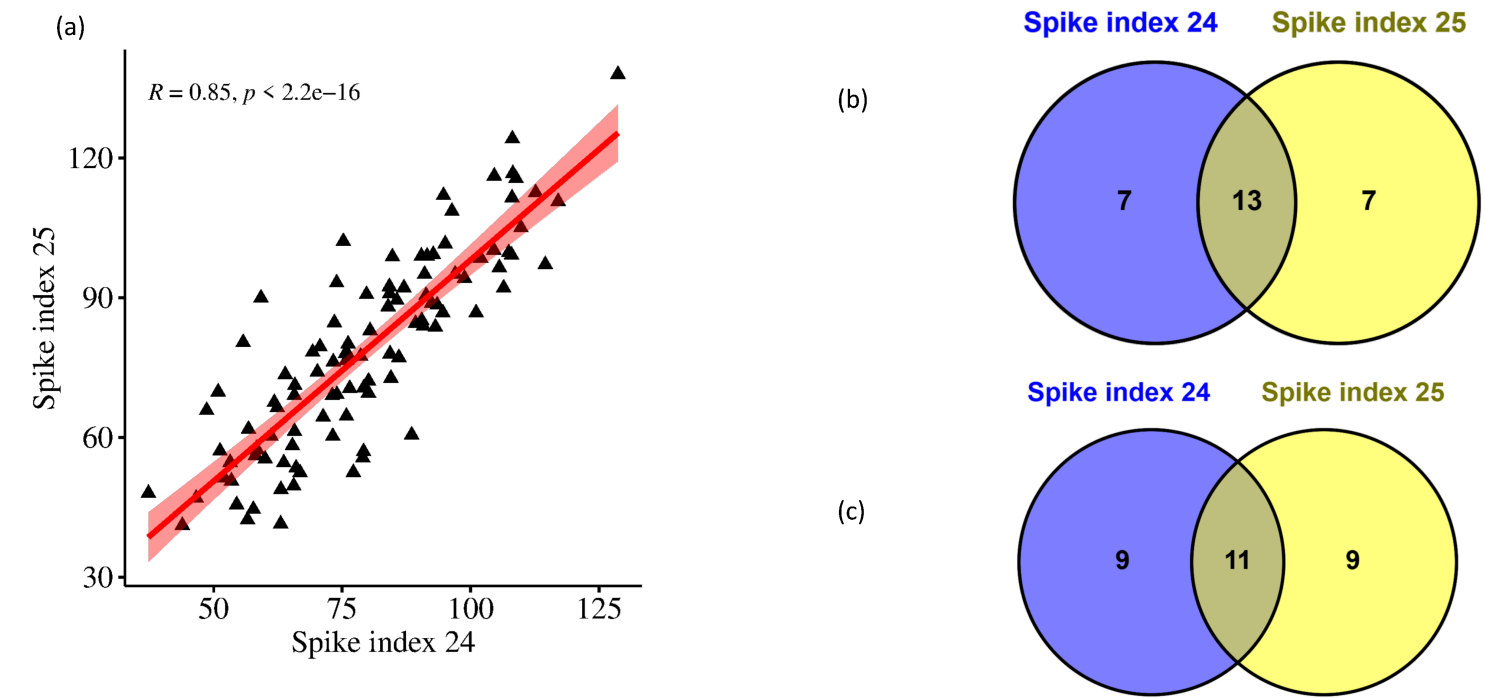


Figure 3: (a) Scatter plot between spike index 24 for first season Spike and spike index 25 for second season (b)Venn diagram common of high genotypes (c)Venn diagram common of low genotypes under heat stress for two seasons.

Figure 3

See image above for figure legend

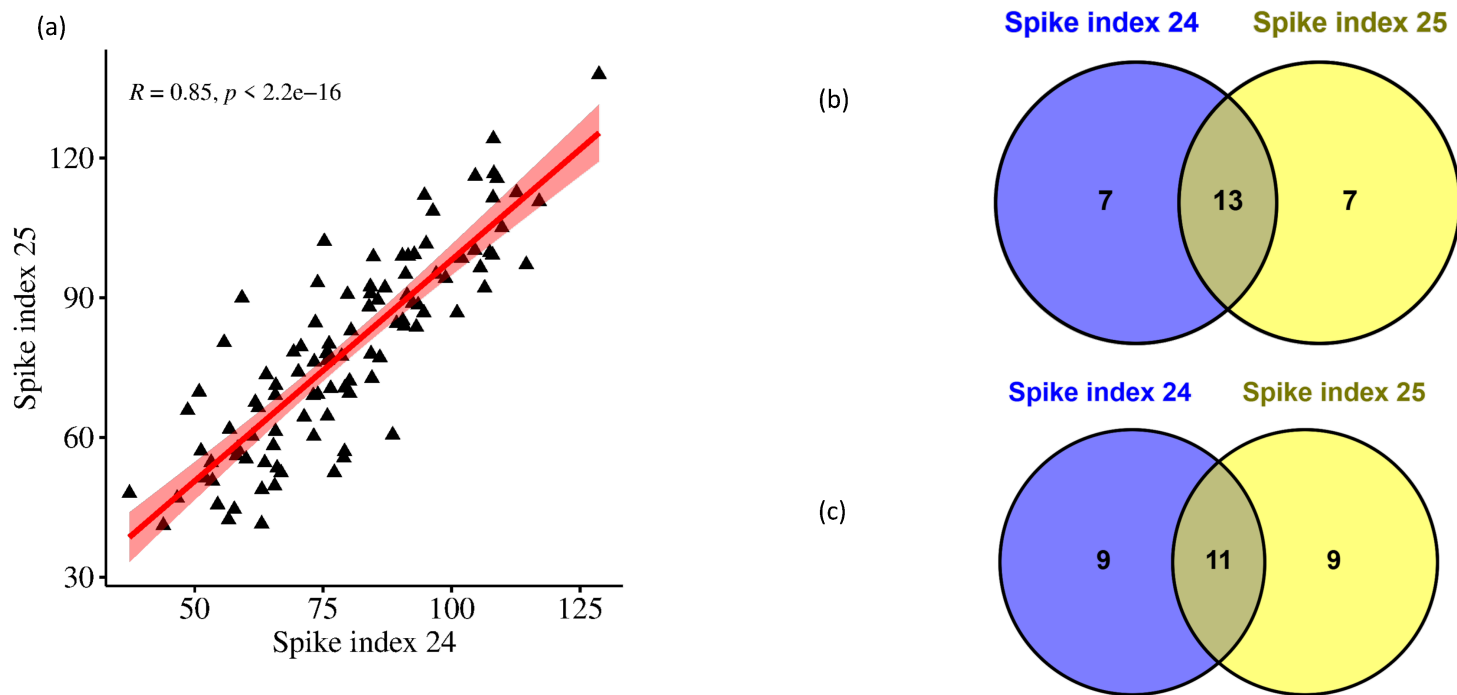


Figure 3: (a) Scatter plot between spike index 24 for first season Spike and spike index 25 for second season (b)Venn diagram common of high genotypes (c)Venn diagram common of low genotypes under heat stress for two seasons.

Figure 3

See image above for figure legend

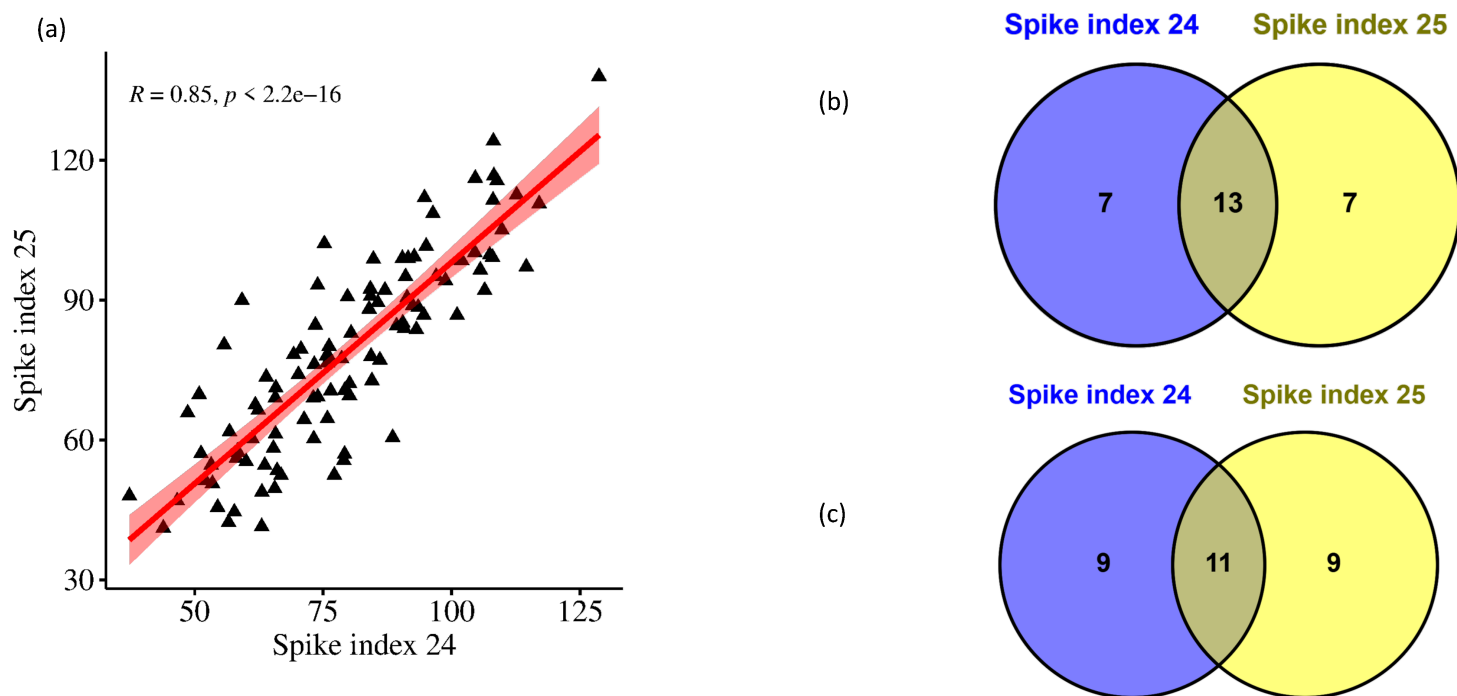


Figure 3: (a) Scatter plot between spike index 24 for first season Spike and spike index 25 for second season (b)Venn diagram common of high genotypes (c)Venn diagram common of low genotypes under heat stress for two seasons.

Figure 3

See image above for figure legend

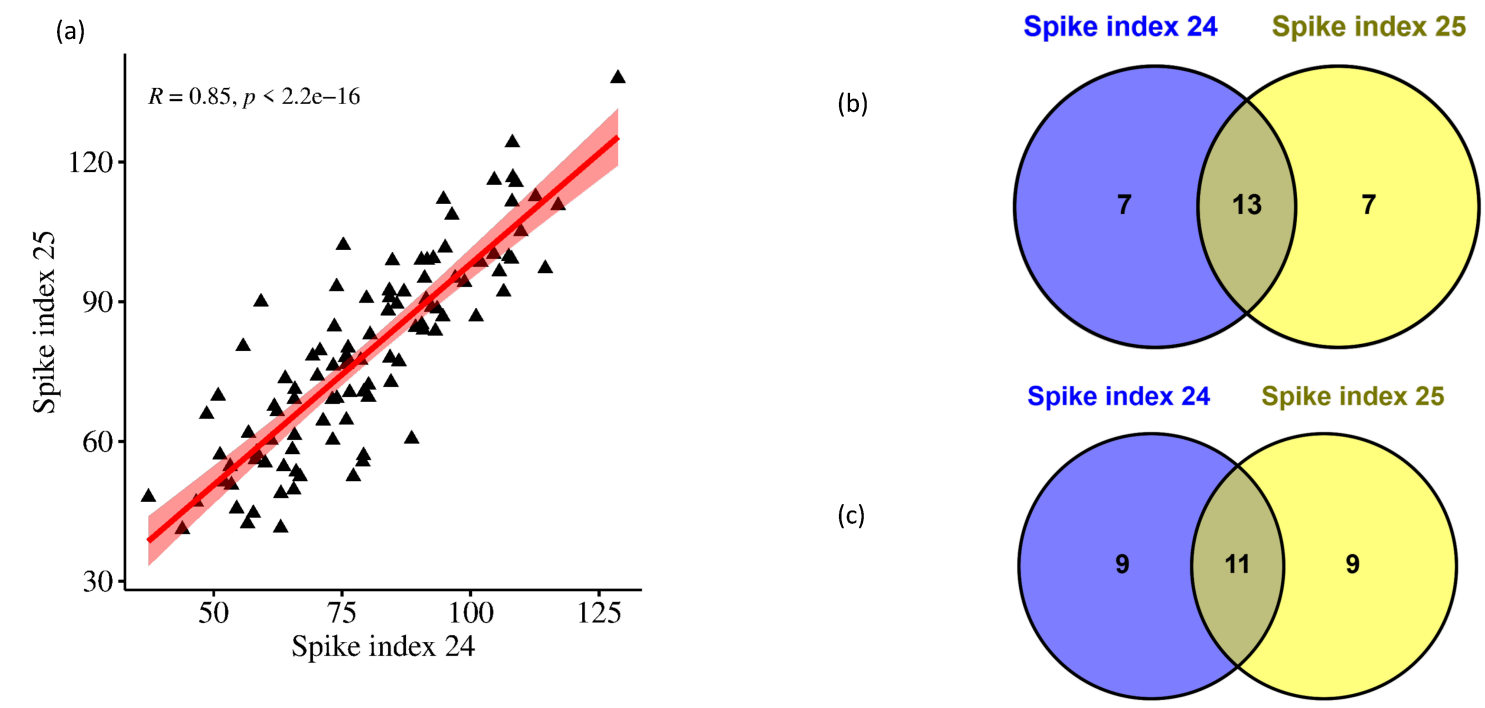


Figure 3: (a) Scatter plot between spike index 24 for first season Spike and spike index 25 for second season (b)Venn diagram common of high genotypes (c)Venn diagram common of low genotypes under heat stress for two seasons.

Figure 3

See image above for figure legend

Figure 4: Spike index (SI) for three subpopulations of genotypes during season 1 (SI24) and season 2 (SI25).

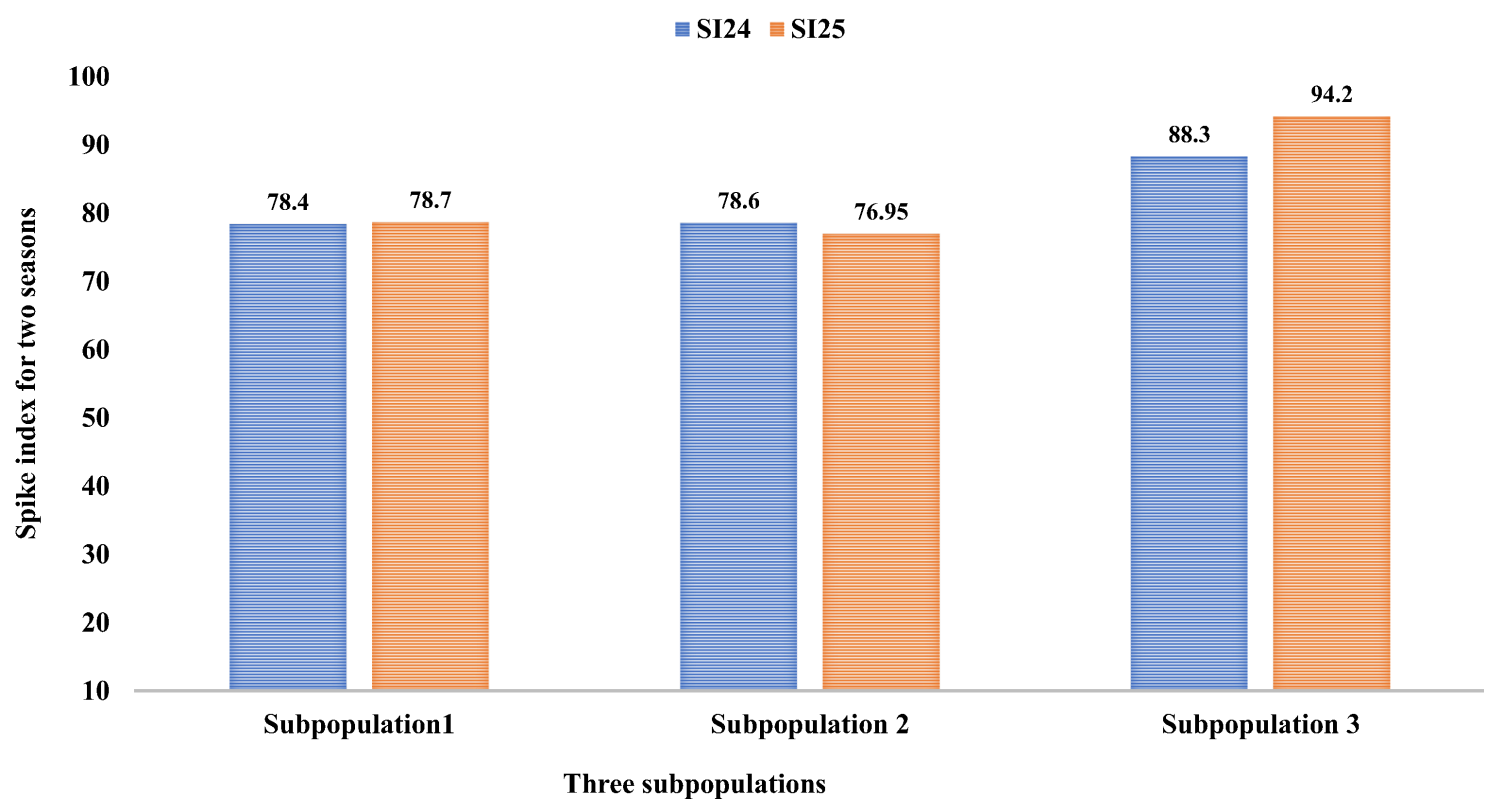


Figure 4

See image above for figure legend

Figure 4: Spike index (SI) for three subpopulations of genotypes during season 1 (SI24) and season 2 (SI25).

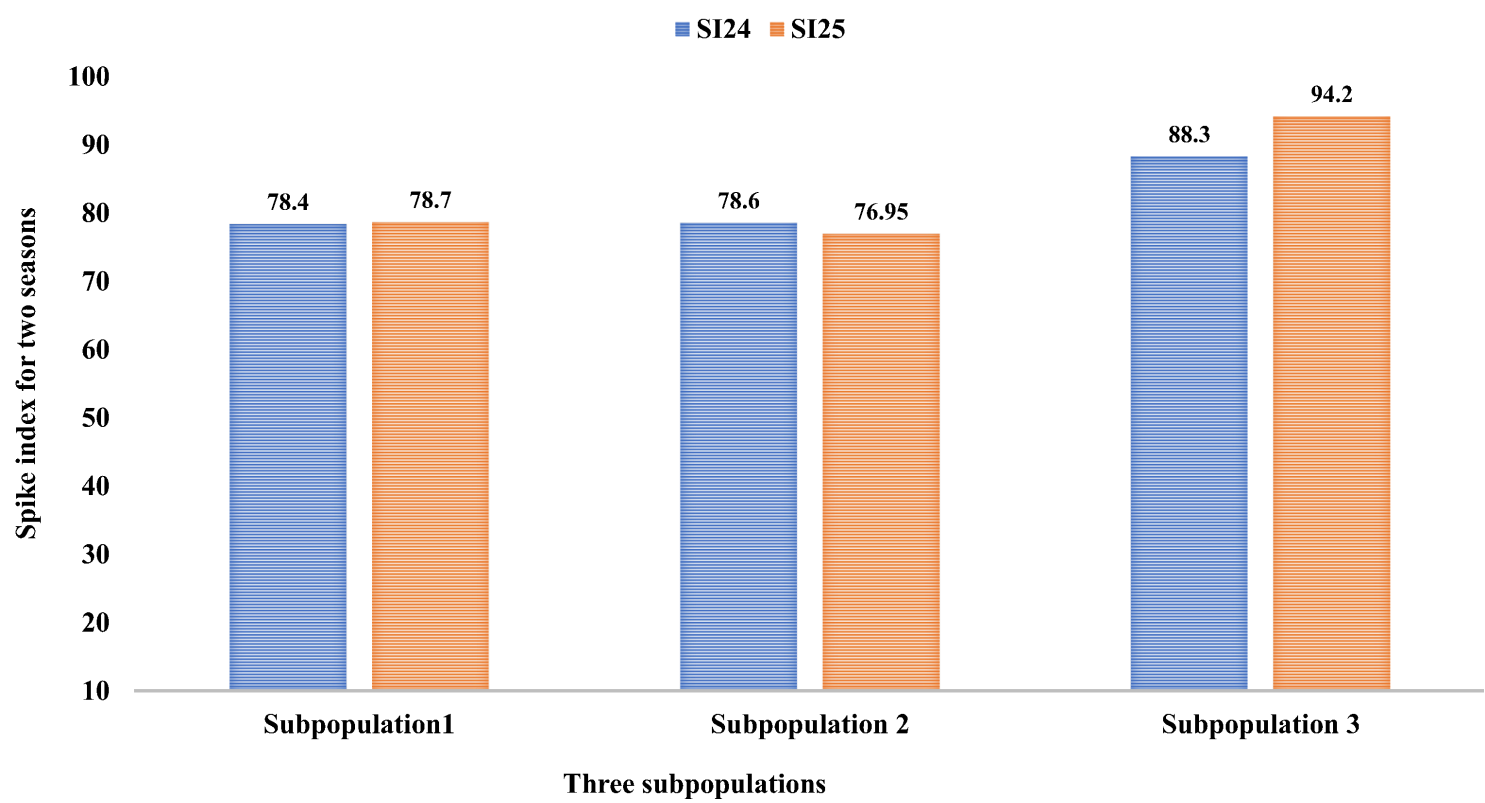


Figure 4

See image above for figure legend

Figure 4: Spike index (SI) for three subpopulations of genotypes during season 1 (SI24) and season 2 (SI25).

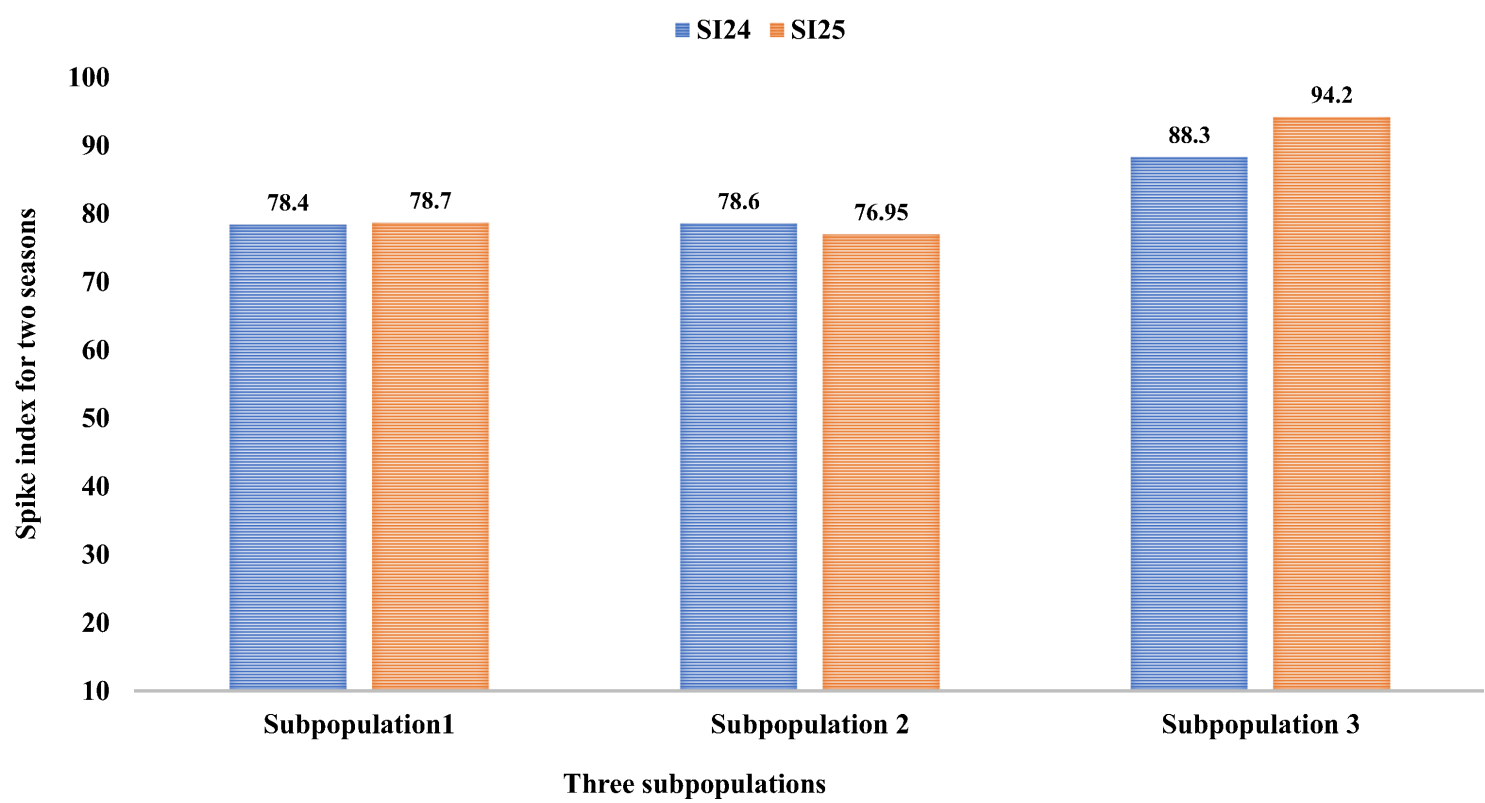


Figure 4

See image above for figure legend

Figure 4: Spike index (SI) for three subpopulations of genotypes during season 1 (SI24) and season 2 (SI25).

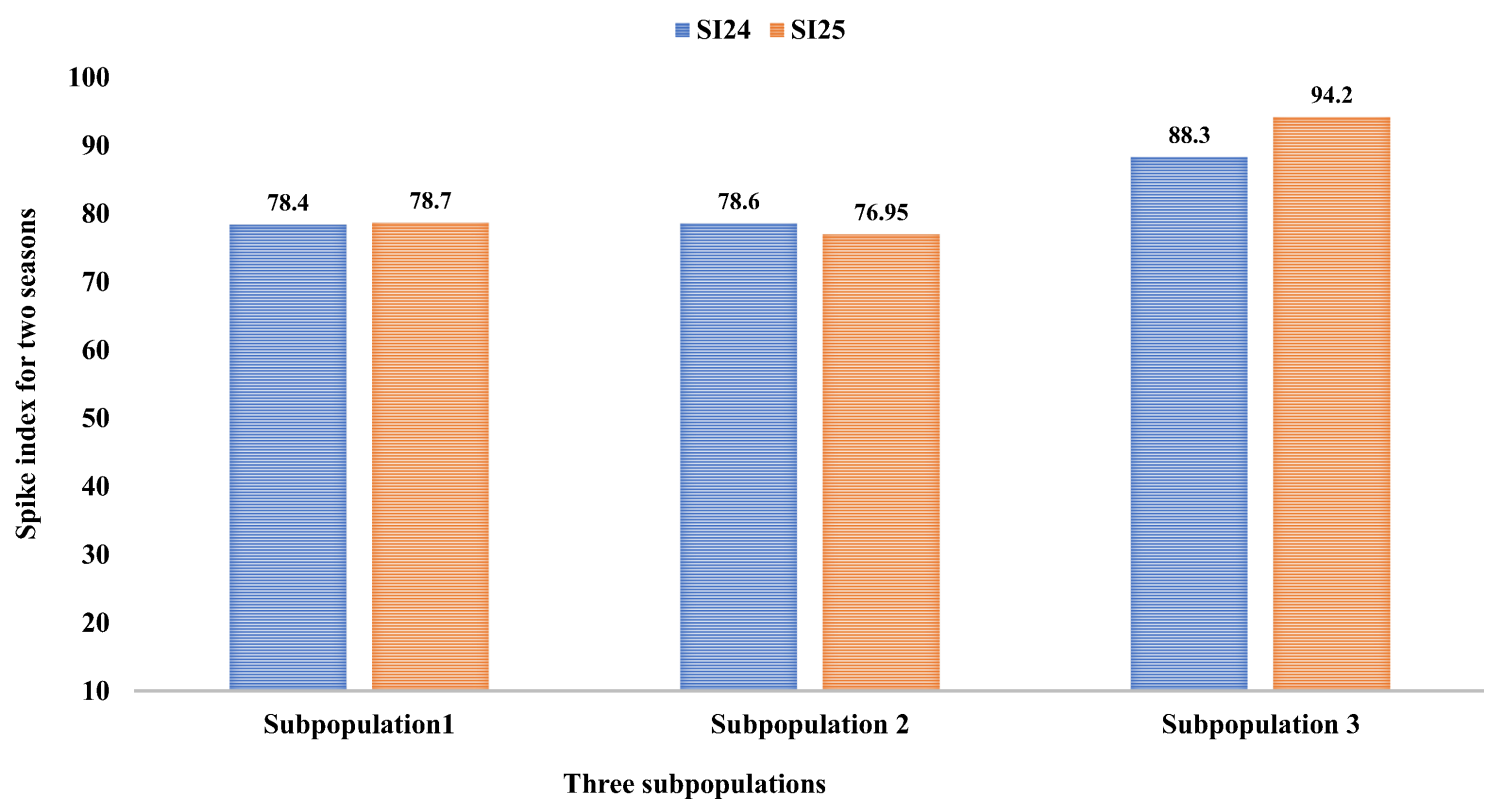


Figure 4

See image above for figure legend

Figure 5: Genetic distance between highly selected genotypes.

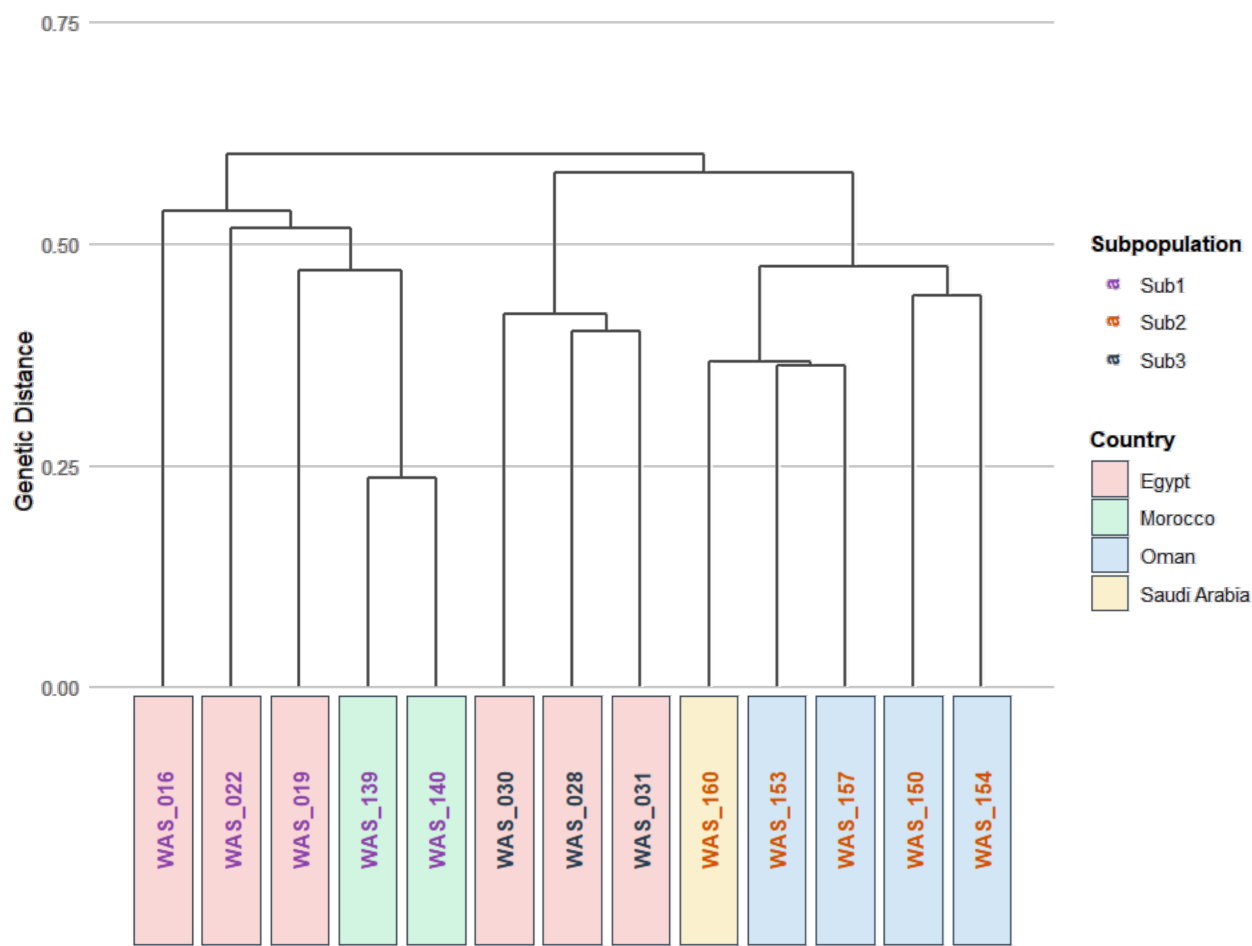


Figure 5

See image above for figure legend

Figure 5: Genetic distance between highly selected genotypes.

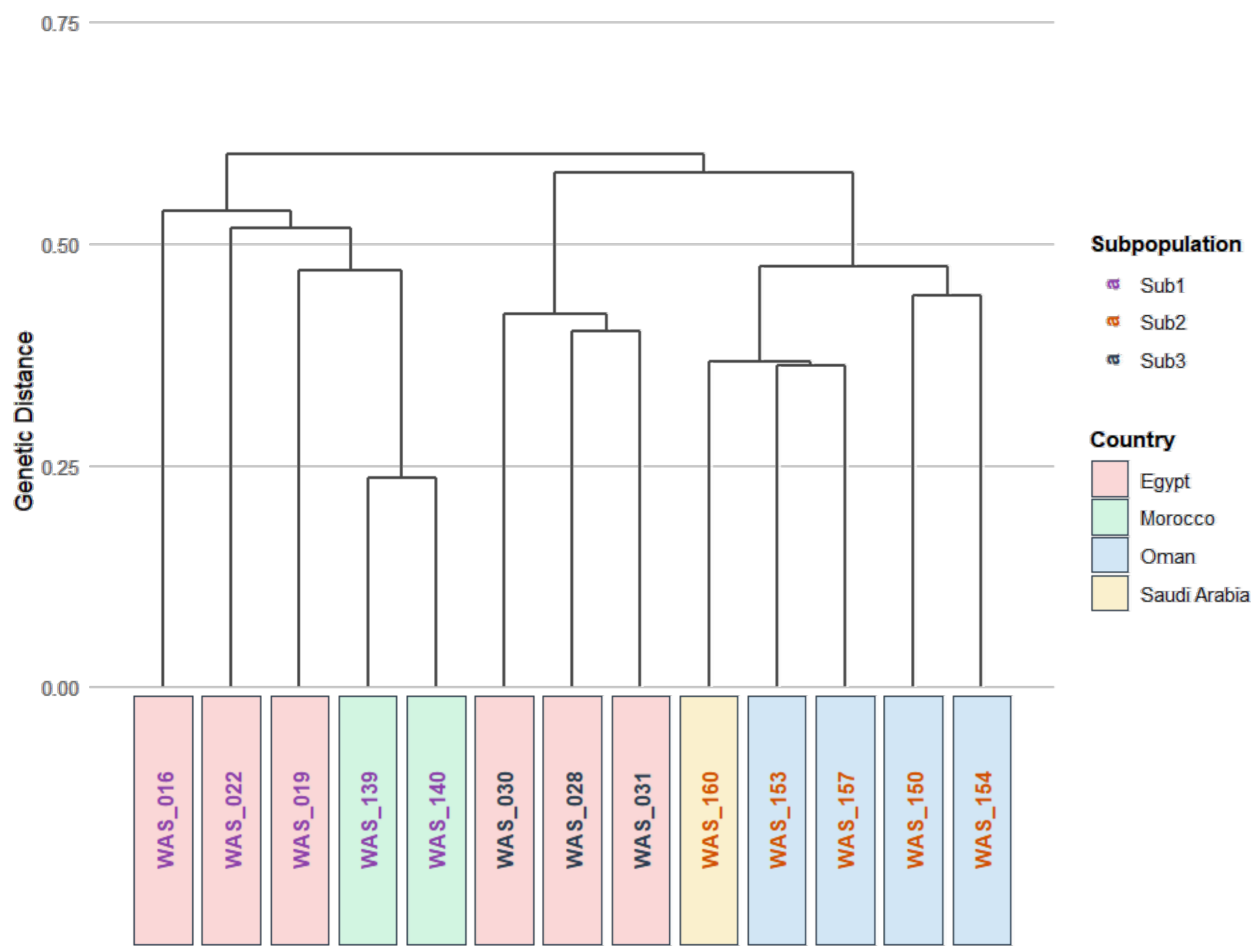


Figure 5

See image above for figure legend

Figure 5: Genetic distance between highly selected genotypes.

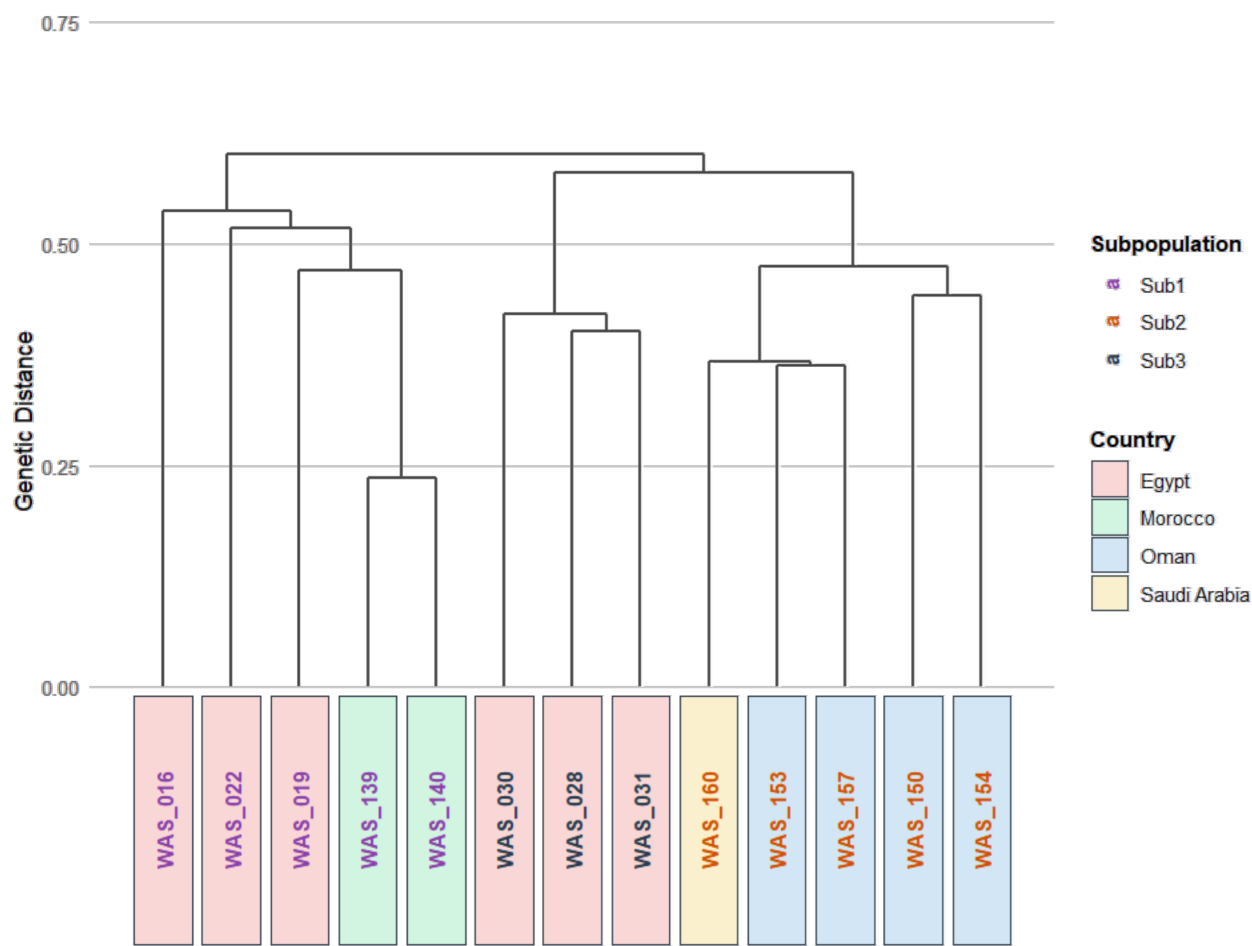


Figure 5

See image above for figure legend

Figure 5: Genetic distance between highly selected genotypes.

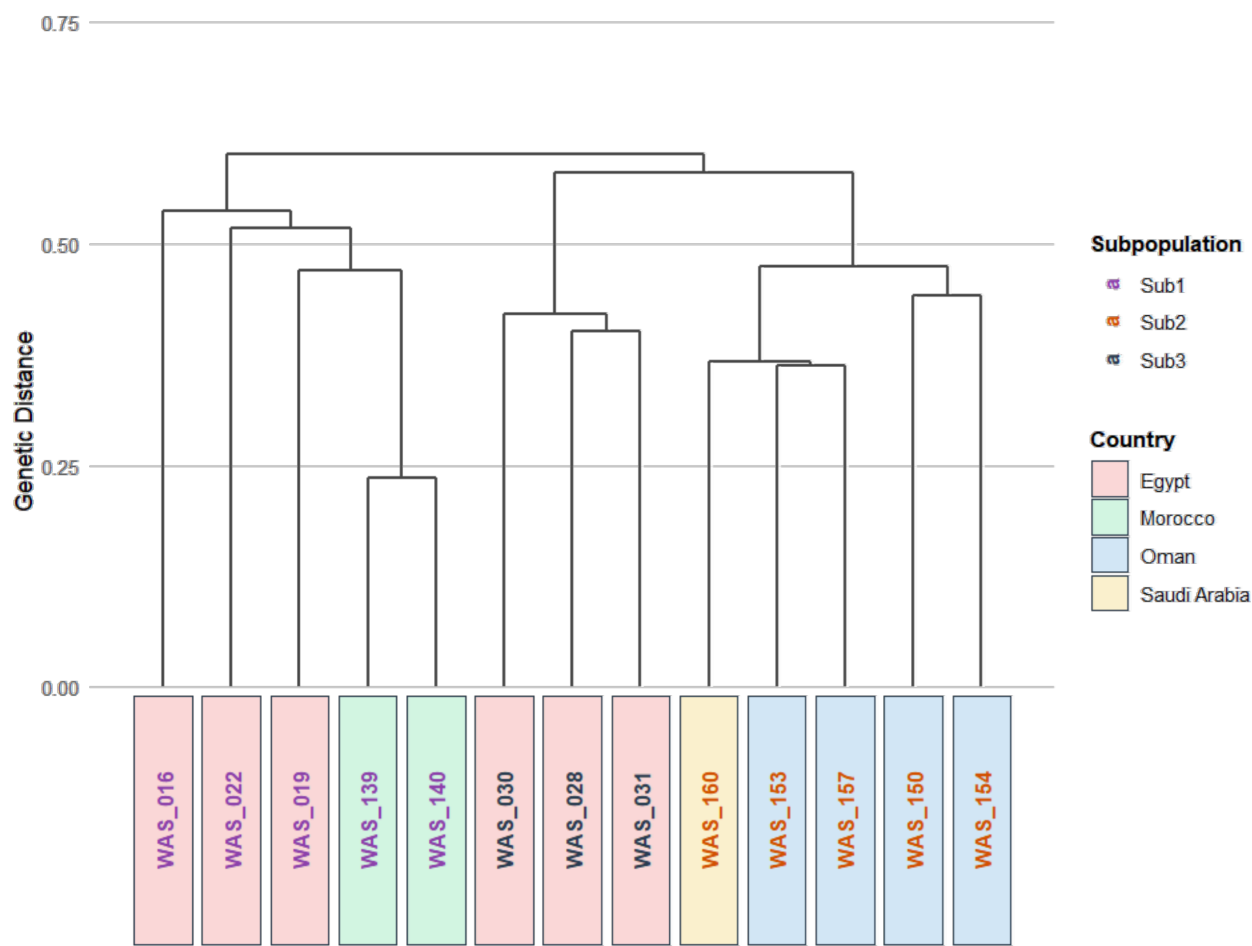


Figure 5

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryfile1.xlsx](#)
- [Supplementaryfile1.xlsx](#)
- [Supplementaryfile1.xlsx](#)
- [Supplementaryfile1.xlsx](#)