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Effect of High Temperature Stress on Pollen Grains in Sunflower (*Helianthus annuus* L.) Inbred Lines

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HIGHLIGHTS

- Pollen grains of ten sunflower inbred lines were evaluated under normal and high temperature stress conditions.
- Two temperature treatments and three treatment times were used as factors
- Pollen viability, pollen germination percentage and pollen tube length decreased with increasing temperature and time period.

Abstract: High temperature at reproductive stage causes a decrease in seed set that ultimately results in yield loss in important crops. Limited research is available regarding the effect of high temperature on viability attributes of pollen grain in sunflower. This study was planned to reveal the effects of high temperature stress on pollen grains in sunflower. An experiment was laid out in a triplicated Completely Randomized Design with split-split plot arrangements at the Oilseeds Research Institute, Ayub Agricultural Research Institute Faisalabad during spring 2021. Two temperature treatments T1 = 45° C for 10, 20 and 30 minutes, and T2 = 50° C for 3, 5 and 10 minutes were used on 10 sunflower inbred lines. Normal (untreated) pollen grains were used as control (T0). Data were collected on pollen viability, pollen germination, and pollen tube length. Recorded data were subjected to analysis of variance, Tukey's HSD mean comparison test, and correlation. According to the results, ORI-1 showed the highest mean for pollen viability, whereas, RL-86 had highest mean for pollen germination and pollen tube length under normal conditions. RL-86 and ORI-73 revealed their ability to withstand both heat stresses as all pollen traits were least affected under high temperature stresses. It is advised to incorporate these inbred lines in future breeding programs aimed at the development of heat tolerant sunflower hybrids. Moreover, correlation studies displayed a positive, strong and highly significant relation of pollen germination with pollen tube length under normal and heat stress conditions revealing their importance in pollen study and reproduction.

Keywords: Abiotic stress tolerance; pollen germination; pollen tube length; pollen viability; sunflower.

INTRODUCTION

Palynology encompasses the study of pollens, spores and microscopic planktons. Studying the characteristics, viability, and germination of plant pollens is essential as it makes up the only part of male gametophytes in plants having direct role in heredity as well as seed development [1]. Sunflower which is the 4th most important edible oil in the world after palm, soybean, and rapeseed oils contributing 19.27 million metric tons (9%) in the total production [2]. Total area under sunflower cultivation during 2021 was more than 28 million hectares with over 50 million tons sunflower seed yield around the globe [3].

Global agriculture is at threat due to global climatic changes [4]. The major concern is the rise in temperature which increases vulnerabilities for the agricultural sector. High-temperature stress is one of the most important abiotic adversities that limit crop growth, development, and productivity. It is considered the greatest threat to food security and agriculture [5]. In developing countries, a 2°-3° C rise in temperature can cause crop yield loss of 20-30% by 2050 [5, 6]. A drop of 30% crop yield is estimated to occur by the end of this century in South Asia alone [7, 8].

The elevated temperature has detrimental effects on plant's morphology, physiology, and biochemical processes. A rise in temperature can cause leaf abscission, senescence, sunburn of branches, shoot, leaf death, and ultimately reduced yield [9, 5]. Other effects include increased respiration rate, decreased photosynthetic activity, sugar and starch contents along with male sterility, and low pollen viability [10]. The reproductive stage in plants holds great importance as it determines the success of seed development and fruit set. However, it is also the most sensitive stage to abiotic stresses especially supra-optimal temperature. The high temperature at the time of reproduction has been linked with impaired fertilization and post-fertilization processes as a result of loss of pollen and ovule viability, failure of pollen germination, and pollen tube growth, transformed stigmatic and style positions [11]. Generally, female gametophyte is tolerant to high temperature as compared with male gametophyte [12].

In male gametophyte, male sterility and impaired pollen development occurring due to heat stress are the major causes of reduced yield [13, 5]. Pollen grain development is extremely sensitive to high-temperature stress as observed by [14] in maize, [15] in cowpea, [16] in chickpea, [13] in rice and [17] in tomato. The effect of heat stress and its damage to male gametophytes can be determined at the microsporogenesis stage [6].

The yield and oil content have always been the focus of sunflower breeders as they are the main breeding goals [18–20]. In sunflower, environmental factors have a great influence on the achene yield as it is a polygenic character. Abiotic stresses especially supra-optimal temperature affect the viability of pollen, thus affecting the size and number of seeds produced by the parent plant [21]. During the months of April and May, sunflower being exposed to high-temperature stress at the reproductive stage results in great loss in yield [22].

Genetic variability comes from pollen because it plays the main role in transferring the genetic material to the next generation [22, 20]. The fertility of pollen holds great importance because it is directly involved in the reproduction of plants. Pollen viability affects achene yield in sunflowers because only they can fertilize and germinate [21]. A great decrease in pollen viability and pollen germination has been observed in many crop species like cotton [23, 24], sorghum [25, 26], wheat [27, 28], rice [29], and soybean [30]. Therefore, researchers are increasingly focusing on studying the impact of global climate change, especially high temperatures and drought on agricultural productivity [5]. As a result, more attention is being paid on cultivating sunflowers under changing climatic condition without affecting its yield and quality.

Keeping in view the above situation, this study was planned to determine the effect of high temperature on pollen grains of sunflower inbred lines. It also involved the selection of potential sunflower inbred lines having better pollen viability, germination percentage and pollen tube length under high-temperature stress. Further, simple correlation analysis was performed to investigate the potential relationship between pollen traits under normal as well as heat stress conditions.

MATERIAL AND METHODS

Collection of germplasm

Experimental material was collected from the Oilseeds Research Institute, Ayub Agricultural Research Institute Faisalabad Pakistan. Research was conducted at the Oilseeds Research Institute, Ayub Agricultural Research Institute Faisalabad during spring 2021. Ten sunflower inbred lines (5 maintainers and 5 restorers) were used in the experiment. The description of these inbred lines is provided in Table 1.

Table 1. Sunflower inbred lines used in the experiment

Name of Inbred Lines Male Fertile (B-lines)	Name of Inbred Lines Restorer (R-lines)
ORI-1	RL-67
ORI-20	RL72
ORI-43	RL-86
ORI-73	RL-114
ORI-90	V214

Layout of experiment

Sunflower inbred lines were grown in the field using triplicate Randomized Complete Block Design. Four lines of 5 meter length per replication of each inbred line were sown on ridges. Row to Row and Plant to Plant distance of 75 cm and 25 cm were maintained, respectively. Standard agronomic practices were followed from sowing to harvesting to maintain a healthy crop stand. The recommended dose of fertilizers i.e 118 kg/ha nitrogen, 85 kg/ha phosphorus, and 62 kg/ha potassium was applied to the crop.

Experiment was conducted under laboratory conditions to determine the effects of high temperature and heat treatment time on pollen grains of sunflower. Pollen grains from 10 sunflower inbred lines were used in a triplicate Completely Randomized Design with split-split plot arrangements. Petri dishes were washed with bleaching powder and dried before using in the experiment. Pollen grains of each inbred line were collected at the time of anthesis in a petri dish. Pollen grains were collected in the polythene bags and were immediately brought to the laboratory after collection. Two temperature treatments and three-time periods were selected. Normal pollen grains were used as control. The heat treatments were placed in the main plot, treatment time was in a subplot and inbred lines were allotted to the sub-subplot.

The pollen grains of 10 different inbred lines were treated at 45° C for 10, 20, and 30 minutes. Then, the temperature was set to 50º C and heat temperature time was 3, 5, and 10 minutes. Each treatment was replicated thrice in completely random order.

T0 (Control) = Fresh pollen of each inbred line

T1 = Temperature of 45º C (high temperature 1 or HT1) was applied to pollen grains for 10 min (treatment time 1 or TT1), 20 min (treatment time 2 or TT2), and 30 min (treatment time 3 or TT3)

T2 = Temperature of 50º C (high temperature 2 or HT2) was applied to pollen grains for 3 min (TT1), 5 min (TT2), and 10 min (TT3)

Data were recorded on the following parameters:

Iodine Potassium Iodide (IKI) test for the determination of pollen viability (%)

Specific cellular contents, compartments, and compounds related to pollen viability were visualized after staining of pollen grains [31]. Where, aniline blue, and acetocarmine stain callose, and chromatin, respectively, potassium iodide (IKI) was successfully used for staining starch in pollen grains. The colorless pollen indicates the absence of the respective cellular content, and thus were considered non-viable [32]. The collected pollen grains were observed using IKI staining technique. According to this method, a drop of 1g of potassium iodide and 0.5g of iodine dissolved in 100mL of distilled water was placed on a clean glass slide containing the pollen grains from each sample. The sample was covered immediately to avoid its contact with oxygen and placed in a petri dish with a moist filter paper for 5 minutes at room temperature. Pollen grains stained dark brown or dark red were considered viable and colorless as non-viable under SWIFT M3300-D microscope. After this, viability was calculated in percentage by counting 100 pollen grains in 5 different microscope fields per inbred line per replication using the following formula:

$$\text{Pollen viability} = \frac{\text{No. of viable pollen grains in the field of the microscope (n)}}{\text{No. of total pollen grains in the field of the microscope (N)}} \times 100$$

Pollen germination test (%)

Pollen grains collected from a single plant per inbred line per replication were observed as followed by [33, 6, 12]. 15 g sucrose, 0.03 g of calcium nitrate [$Ca(NO_3)^2$ (6x10-3 M)], and 0.01 g of boric acid [H_3BO_3 ($2x10^{-3}$ M)] were dissolved in 100 mL of deionized water to prepare the germination media. A drop of the

prepared media was placed on a glass slide containing the sample pollen grains. The glass slide was covered immediately and placed in a petri dish with moist filter paper at room temperature for 2 hours. Then, the slides were observed under SWIFT M3300-D microscope. The pollen tubes were considered germinated when the length was equal or greater than the pollen diameter. Pollen germination percentage was calculated using the formula,

$$\text{Pollen germination\%} = \frac{\text{No. of germinated pollen in the field of the microscope (n)}}{\text{No. of total pollen grains in the field of the microscope (N)}} \times 100$$

Pollen tube length (µm)

Length of 25 pollen tubes was observed in 5 different fields of microscope per inbred line per replication with the help of ocular micrometer and their average was calculated.

Statistical analysis

The data were analyzed for variance following the procedure of [34]. The means of inbred lines were compared using Tukey's HSD test. Correlation coefficient analysis among pollen traits under normal and heat stress conditions was carried out as suggested by [35].

RESULTS

All inbred lines under study showed significant differences for pollen germination and pollen tube length under normal experimental conditions (T0). However, a non-significant difference was observed for pollen viability [(Table 2(a)]. Further, Tukey's HSD mean comparison test [Table 2(b)] revealed that RL-86 had the highest pollen germination percentage followed by ORI-73. However, the lowest pollen germination was observed in ORI-43. Longest pollen tube was found in RL-86 following ORI-73. The shortest pollen tube length was observed in ORI-90 under control [Table 2(b)].

Table 2 (a). Analysis of variance of pollen traits in sunflower inbred lines under normal conditions

SOV	DF	PV	PG	PTL
Genotypes	9	15.426 NS	115.984**	492.784**
Error	20	11.826	17.864	16.682
CV	-	3.64	7.84	7.52

NS = non-significant, * and ** denotes significant and highly significant results at probability levels of 5% and 1%, respectively. **SOV =** Sources of variation**, DF =** Degrees of freedom**, PV =** Pollen viability**, PG =** Pollen germination**, PTL =** Pollen tube length

Table 2 (b). Tukey's HSD mean comparison test for pollen germination and pollen tube length in sunflower inbred lines under normal conditions

Genotypes	Means PG	Means PTL
RL-86	62.863^{a}	77.76^{a}
ORI-73	61.83^{ab}	74.93^{a}
RL-72	58.61^{abc}	60.598^{b}
ORI-43	57.253^{abc}	51.771^{bc}
ORI-1	53.263^{abcd}	49.532^{bcd}
RL-67	53.143^{abcd}	48.63^{cd}
V214	51.52^{abcd}	47.437^{cd}
RL-114	49.827^{bcd}	47.285^{cd}
ORI-90	47.143^{cd}	46.893^{cd}
ORI-20	43.78^{d}	38.48^{d}

Different letters in the same column indicate highly statistical differences as per Tukey's test ($P \leq 0.05$)

Pollen study under heat stress

Pollen Viability

Significant differences existed for heat treatment factor, however, factors treatment time and genotypes were found to be highly significant at 1% significance level. All interactions between factors had highly significant differences [Table 3(a)]. Pollen viability was found maximum at TT1 (74.52%) and reduced to

47.37% at TT3. Maximum pollen viability was found in RL-86 followed by ORI-43. Minimum pollen viability was found in RL-72 which showed its sensitivity to heat stress. The interaction effects between heat treatment and treatment time indicated that HT2 at TT3 reduced the pollen viability to 45.25%. RL-86 and ORI-73 at TT1 retained maximum pollen viability as 86.76% and 86.07%, respectively. On the other hand, RL-72 at TT3 had the lowest pollen viability of 21.71%. The interaction effects of heat treatment with genotypes indicated that HT1 had the lowest impact on the pollen viability of RL-86. HT1 and HT2 had the most severe impact on RL-72 as the pollen viability decreased to 31.53% and 36.64%, respectively (Figure 1).

Table 3 (a). Analysis of variance of pollen traits under heat stress conditions split-split plot arrangement

SOV	DF	PV	PG	PTL
Reps	2	7.3	21.2	2.69
Heat Treatment (A)	1	142.3*	781.2**	311.15*
Error	2	3.2	5.6	3.75
Treatment Time (B)	2	11057.2**	11001**	7500.7**
A*B	2	155.8**	3.4	15.36**
Error	8	6	6	1.76
Genotypes (C)	9	3879.7**	879.3**	947.78**
A*C	9	358.2**	154.3**	27.23*
B*C	18	224.3**	81.3**	125.31**
A*B*C	18	138.2**	76.2**	39.1**
Error	108	12.5	9.6	12.34

NS = non-significant, * and ** denotes significant and highly significant results at probability levels of 5% and 1%, respectively. **SOV =** Sources of variation, **DF =** Degrees of freedom, **SS =** Sum of Squares, **MS =** Mean squares, **Fcal =** F-calculated

Table 3 (b). Tukey HSD All-Pairwise Comparisons Test of pollen viability, pollen germination, and pollen tube length for heat treatments

Factor	PV	PG	PTL
HT1	61.956[a]	31.063[a]	36.185[a]
HT2	60.177[b]	26.896[b]	33.555[b]

Different letters in the same column indicate highly statistical differences as per Tukey's test (P ≤ 0.05), HT1: 45°C, HT2: 50°C

Table 3 (c). Tukey HSD All-Pairwise Comparisons Test of pollen viability, pollen germination, and pollen tube length for treatment times

Factor	PV	PG	PTL
TT1	74.519[a]	41.575[a]	45.839[a]
TT2	61.306[b]	30.705[b]	35.281[b]
TT3	47.373[c]	14.659[c]	23.489[c]

At 45°C, TT1: 10 mins, TT2 : 20 mins, TT3 : 30 mins. At 50°C, TT1 : 3 mins, TT2 : 5 mins, TT3 : 10 mins

Pollen Germination percentage

The analysis of variance for pollen germination is given in Table 3(a). HT1 and HT2 reduced the pollen germination percentage of inbred lines to 31.06% and 26.89%, respectively [Table 3(b)]. Pollen germination was maximum at TT1 (41.57%) and reduced to 14.65% at TT3 [Table 3(c)]. The results of the interaction effects indicated that HT2 at TT3 reduces the pollen germination to 12.83%. RL-86 and ORI-73 at TT1 retained maximum pollen germination of 57.17% and 54.91%, respectively. On the other hand, V214 at TT3 had the lowest pollen germination percentage of 4.86. The interaction effects of heat treatment with genotypes indicated that HT1 had the lowest impact on the pollen germination of ORI-73 following RL-86. HT2 had the most severe impact on V214 as the pollen germination decreased to 13.78%. According to the interaction effects between all three factors, RL-86 at HT2 TT1 retained maximum pollen germination of 58.2% following ORI-73 and RL-86 at HT1 TT1. ORI-90 showed zero pollen germination at HT1 TT3, however, RL-72 and V214 also had zero germination at HT2 TT3 (Figure 2).

Table 3(d). Tukey HSD All-Pairwise Comparisons Test of pollen viability, pollen germination, and pollen tube length for genotypes

Factor	PV	PG	PTL
RL-86	83.405[a]	41.536[a]	50.703[a]
ORI-43	76.834[b]	40.802[a]	45.018[b]
V214	69.993[c]	30.246[b]	35.655[c]
RL-114	68.950[c]	29.496[b]	33.112[cd]
ORI-20	62.799[d]	28.332[b]	32.602[cd]
ORI-73	62.275[d]	24.972[c]	31.769[de]
ORI-90	51.217[e]	24.643[c]	31.477[de]
RL-67	50.926[e]	24.588[c]	30.322[de]
ORI-1	50.168[e]	24.031[cd]	29.771[de]
RL-72	34.098[f]	21.151[d]	28.269[e]

Pollen Tube Length

The results indicated that all three factors and their interactions were observed to be highly significant at 1% significance level indicating the existence of a high amount of variability among genotypes for this trait [Table 3(a)]. Pollen tube length of inbred lines reduced from 36.19 µm at HT1 to 33.56 µm at HT2 [Table 3(b)]. Pollen tube length was maximum at TT1 (45.84 µm) and reduced to 23.49 µm at TT3. The interaction effects indicated that HT2 at TT3 reduces the pollen tube lengths to 22.06 µm. ORI-73 and RL-86 at TT1 retained maximum pollen tube lengths of 65.06 µm and 63.407 µm, respectively. The interaction effects between heat treatments and genotypes indicated that HT1 and HT2 had the lowest impact on the pollen tube length of RL-86. HT2 had the most severe impact on ORI-90 as the pollen tube length decreased to 27.34 µm. Interaction effects of all three factors showed that ORI-73 and RL-86 at both heat treatments with TT1 retained maximum pollen tube lengths of 67.41 µm and 65.87 µm. However, RL-114, ORI-90, and RL-72 were the most affected at HT1 TT3, and HT2 TT3, respectively. In these inbred lines, pollen tube length was found to be smaller than the diameter of pollen grains (Figure 3).

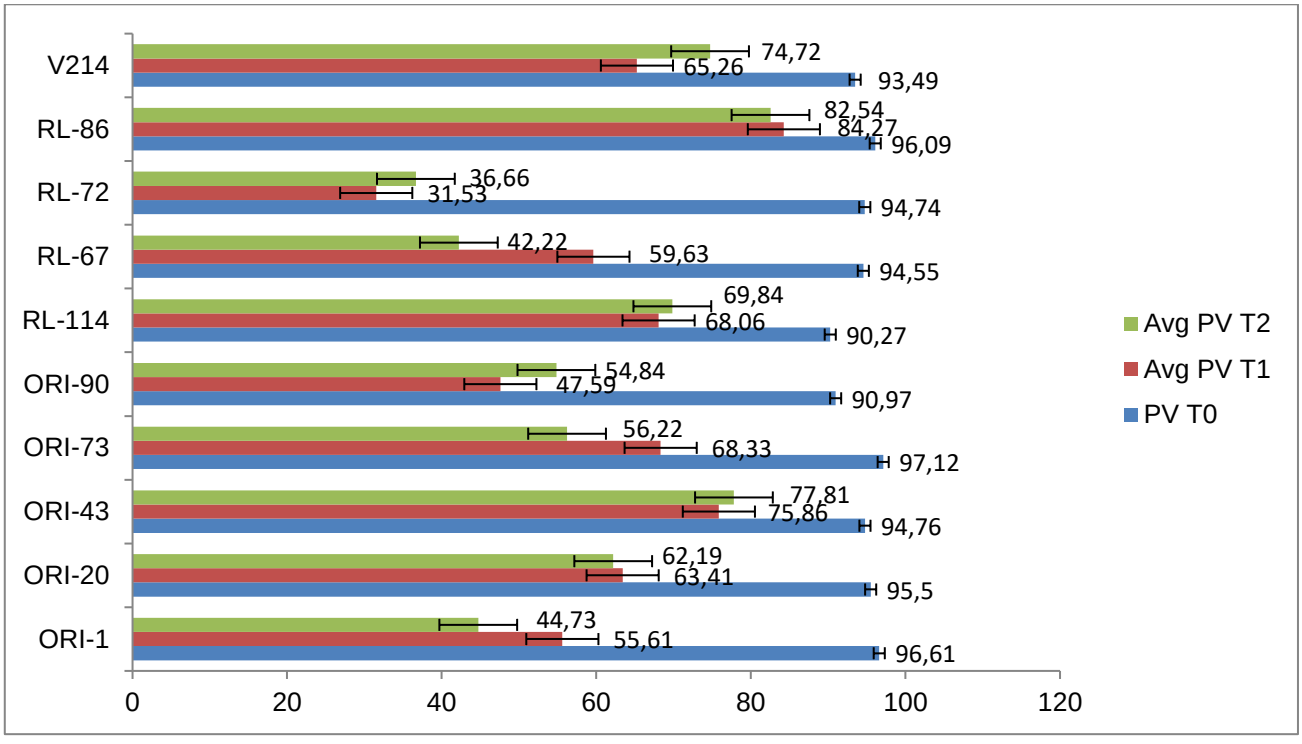

Figure 1. Pollen viability percentage of sunflower inbred lines under control and heat stress conditions. Average values of different treatment times per heat treatment were used.

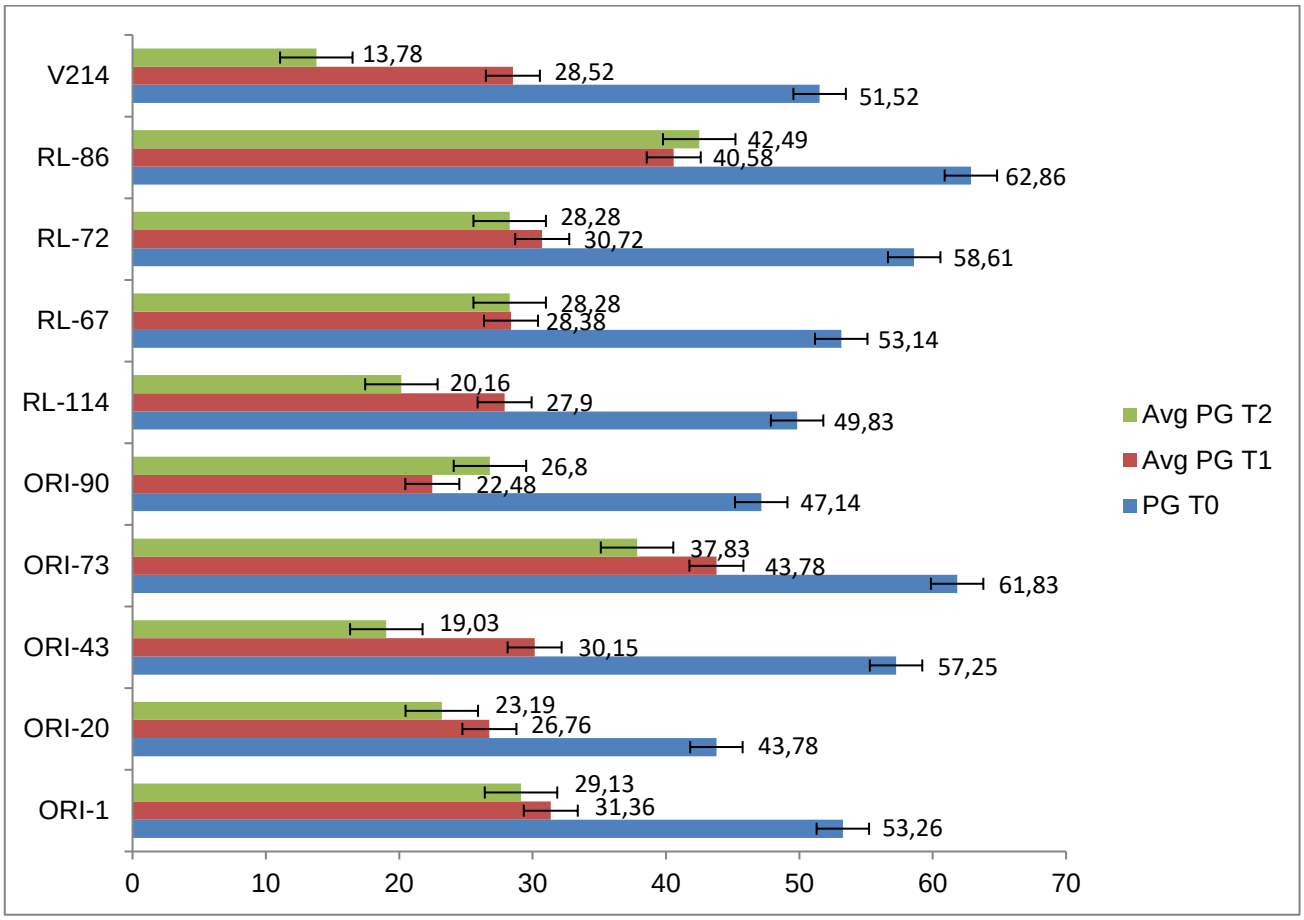

Figure 2. Pollen germination percentage of sunflower inbred lines under control and heat stress conditions. Average values of different treatment times per heat treatment were used.

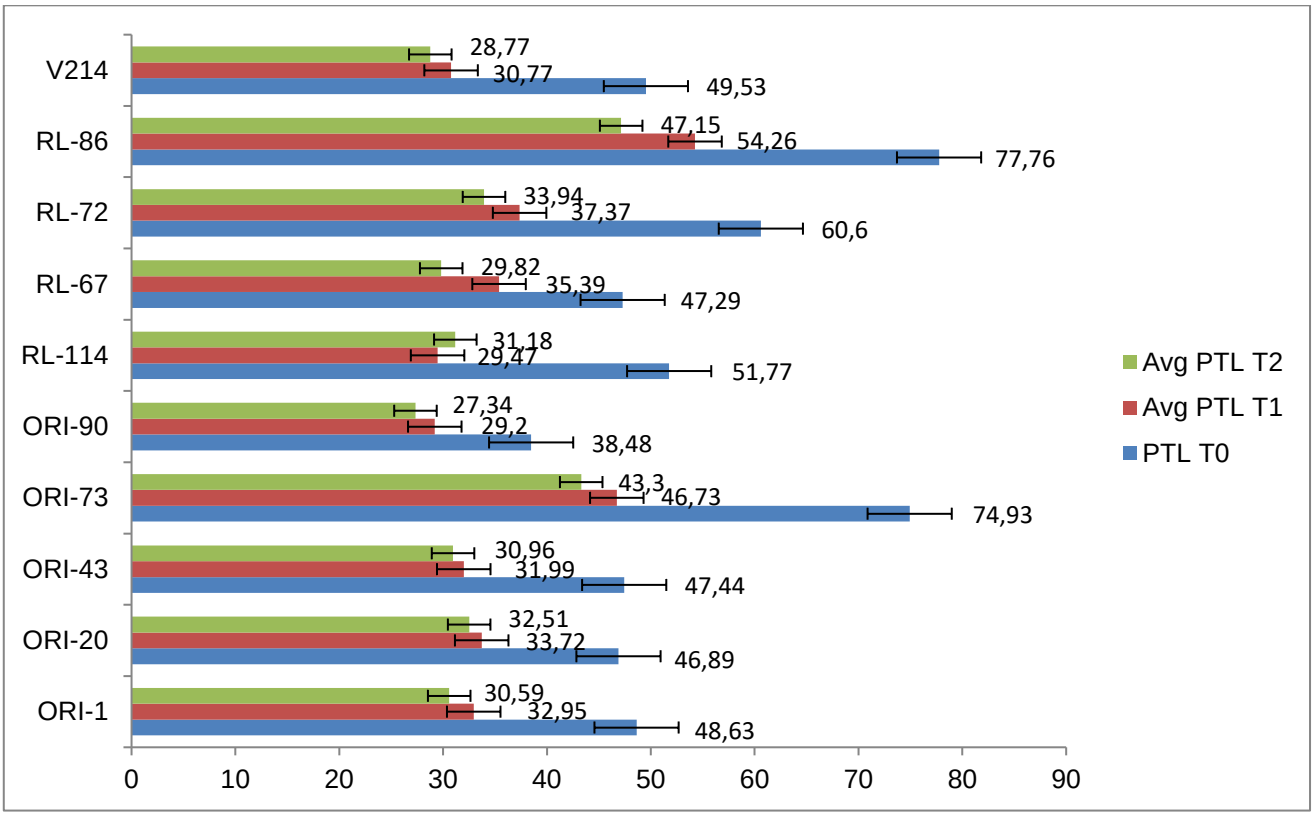

Figure 3. Pollen tube length of sunflower inbred lines under control and heat stress conditions. Average values of different treatment times per heat treatment were used.

Correlation Coefficient Analysis

The results for correlation coefficient analysis between pollen traits are presented in Table 4. Pollen viability under normal conditions (T0) showed positive, moderate but non-significant relationship with pollen germination (T0) and pollen tube length (T0). However, pollen viability (T0) was found to have positive, significant but moderate association with pollen germination (T1) and pollen tube length (T1). It was also found that pollen germination under normal (T0) as well as both heat stress conditions (T1; T2) was positively, strongly and highly significantly associated with pollen tube length under normal and heat stress conditions (Figure 4).

Table 4. Correlation coefficient analysis for pollen traits under normal and heat stress conditions

	PV T0	PV T1	PV T2	PG T0	PG T1	PG T2	PTL T0	PTL T1
PV T1	0.1926							
PV T2	-0.1357	0.8333**						
PG T0	0.544	0.2534	0.0928					
PG T1	0.693*	0.4524	0.1828	0.8477**				
PG T2	0.5411	0.1405	-0.1527	0.6258*	0.7319**			
PTL T0	0.5458	0.3557	0.1978	0.8269**	0.9403**	0.7469**		
PTL T1	0.6302*	0.4034	0.1831	0.7725**	0.8865**	0.8722**	0.9294**	
PTL T2	0.5742	0.468	0.2825	0.7465**	0.9165**	0.8202**	0.961**	0.969**

PV: pollen viability, PG: pollen germination, PTL: pollen tube length

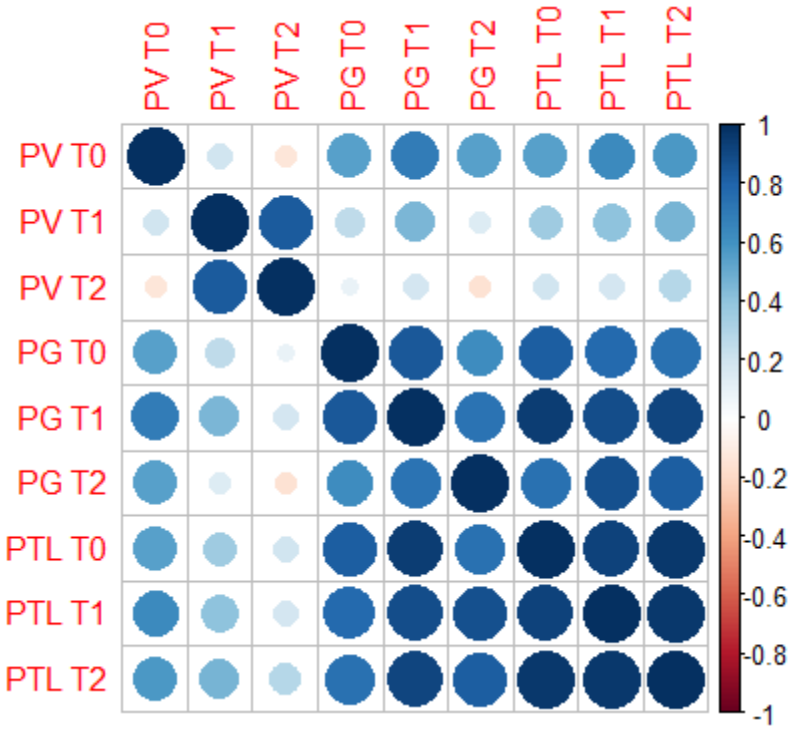

Figure 4. Correlation coefficient analysis of pollen traits

DISCUSSION

Palynology is the study of pollen characteristics, viability, and germination. Pollen study is essential as it provides the basic knowledge for breeding and hybridization [1]. Despite its great importance, it is the least touched area in sunflower. It is necessary to have information about the genetic variability for high-temperature tolerance in the existing sunflower germplasm [5]. For sustainable agriculture, it is important to develop sunflower cultivars that can tolerate high temperatures without compromising the yield. Successful reproductive development is necessary for not just the production of seed and food but also the future improvement of plant breeding depends on it. The assessment of pollen viability and in vitro pollen

germination is an essential tool to determine flowering period, life cycle, genetics and the ability of pollen grains to develop pollen tubes inside style for the release of two sperm cells in female gametophyte.

At optimum temperature, 73% pollen viability in soybean [30], 85% in sorghum [12], almost 100% in rice [14] are reported. Sulusoglu and cavusoglu, 2014 [36] checked the pollen viability of cherry laurel using 2 different staining methods, TTC and IKI, and found 92.4% and 94.1% pollen viability by respective methods indicating that both techniques show similar results. In our study, the results of pollen viability under heat stress clearly showed that RL-86 can withstand short-term high and long-term mildly high temperatures, following V214 and RL-114. Pollen viability decreased from 73% at OT (optimum temperature) to 56% at HT in soybean [30], 85% at OT of 30/20°C to 61% at HT1 (36/26°C) and 20% at HT2 (39/29°C) in sorghum [12], 100% at OT to 56% at HT of 40°C in maize [14]. Pollen viability was found to be highly correlated with grain yield [37], and damages to pollen morphology caused by HT during pollen development lead to the reduction in pollen viability percentage [38].

Nguyen and coauthors, 2013 [39] observed around 70% pollen germination in sorghum under normal conditions. Around 71% of pollen germination was reported in sorghum [12], 20-60% in cotton [40], 60-70% in cherry laurel [36]. Impe and coauthors, 2020 [31] also found the percentage of in vitro pollen germination lower than pollen viability because the former was greatly varied under different growth mediums and conditions. They suggested that a perfect in vitro pollen germination media should be optimized for each species to determine these pollen characters correctly. The results of our in vitro pollen germination study under heat stress indicated that RL-86 has the ability to withstand short-term high and long-term mildly high temperatures, following ORI-73 which showed tolerance to long-term mildly high temperatures. These inbred lines showed the potential to be incorporated in future studies aimed at the development of high-temperature tolerant hybrids. Nguyen and coauthors, 2013 [39] reported 17-63% pollen germination at HT stress of 38°C in sorghum contrasting the pollen germination of 70-80% at the optimum temperature of 32°C. He also found a positive correlation between pollen germination and seed set. Pollen germination decreased from 71% at OT (30/20°C) to 52% at HT1 (36/26°C), and 12% at HT2 (39/29°C) in a study conducted in sorghum by [12]. Zero pollen germination was found at 10°C and 45°C in cotton [23], 20% decrease in rice at 32°C [10]. A decreasing trend in pollen germination with increasing temperature in cotton was observed by [24], however, it is found that anatomical changes in pollen grains are associated with decreased pollen germination at HT stress [30].

The results on pollen tube length under heat stress displayed ORI-73 and RL-86 capable of withstanding short-term high and long-term mildly high temperatures. Therefore, these inbred lines can to be exploited in a hybrid breeding. A similar decrease in pollen tube length with increasing temperature in rice was reported by Das and coauthors, 2014 [29]. It is stated that genotypes with higher pollen tube lengths and pollen germination resulted in a higher boll retention rate in cotton [23].

According to correlation analysis, pollen germination was found to have positive and strong association with pollen tube length under normal and heat stress conditions. We conclude that selection of these traits would improve sunflower productivity even under heat stress. Although, further study is suggested to fully understand the relationship between pollen traits.

CONCLUSION

The study revealed that ORI-1 had the highest mean for pollen viability under normal conditions, while RL-86 followed by ORI-43 maintained maximum pollen viability under long term high (T1) and short term high (T2) temperature treatments. Long term high temperature had the most damaging effect on RL-72 and ORI-90 causing 70% and 63% decrease in pollen viability, respectively. RL-67 lost 76% and RL-72 lost 75.6% pollen viability under short term high temperature treatment. Pollen germination percentage under normal as well as both heat treatments was highest in RL-86 and ORI-73 indicating their excellent ability to perform well under heat stress conditions. However, ORI-90, V214 and RL-72 had zero pollen germination at T1 and T2, respectively. Highest mean for pollen tube length under normal as well as heat stress conditions were found in RL-86 and ORI-73 whereas, lowest was found in ORI-90, RL-114, and RL-72. Positive, excellent and highly significant association between pollen germination and pollen tube length exhibited core importance of these traits in pollen study and reproductivity in sunflower. However, successful in vitro germination test largely depends on an optimum media for different species. In our results, pollen tubes didn't reach their maximum length even under normal conditions because of the unavailability of an optimum media for the determination of in vitro pollen germination in sunflower. It is suggested that further research is required to estimate in vitro pollen germination of sunflower pollen grains using different growth media to optimize a perfect media to study this character.

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