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Temperature Regime Impacts on Reproductive Performance in Various Chickpea (*Cicer arietinum* L.) Genotypes

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ABSTRACT

Background: Early flowering stages with low temperatures (0 to 10°C) may also result in increased floral abortion, which lowers pod and seed set. Both the duration and rate of grain filling are sensitive to temperature changes and high temperatures may reduce productivity due to decreased flowering, pod formation and seed set, which could result in a loss of output. To mitigate the effects of high or low temperatures on chickpea productivity, it is crucial to investigate pollen traits, including pollen fertility, sterility, germination, tube length and diameter.

Methods: The field experiments were conducted at Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad during *rabi* 2018-19 and 2019-20 using six chickpea genotypes with three replications. Sowing dates *i.e.,* Early sown (40th Standard Meteorological Week *i.e.,* 5th October), normal sown (45th Standard Meteorological Week *i.e.*, 5th November) and late sown (49th Standard Meteorological Week *i.e.,* 5th December) of *rabi* season during 2018-19 and 2019-20 were manipulated in order to create the effects of high temperature.

Result: Early-sown chickpea genotypes (planted in October) experienced low-temperature stress, while late-sown genotypes (planted in December) were subjected to high-temperature stress. The late sowing period led to poor performance due to high-temperature exposure, negatively impacting flowering, pod formation and seed set, ultimately lowering the productivity of the chickpea crops. Among the genotypes, positive correlation was seen between maximum temperature and pollen tube length and sterile pollen diameter during 2018-19 (0.770**, 0.545*) and 2019-20 (0.548*, 0.542*) and the average of two years (0.543**, 0.633**), respectively in JAKI 9218 genotype. This relationship was consistent across both years of the study (2018-19 and 2019-20), indicating that increased temperatures may influence pollen fertility and sterility, thereby affecting chickpea productivity.

Key words: Correlation, Pollen diameter, Pollen fertility, Pollen germination.

INTRODUCTION

This study investigates how different temperature regimes affect the reproductive outcomes of various chickpea genotypes. By assessing phenological stages, flower and pod development, seed set and overall yield under controlled temperature conditions, we aim to identify temperature thresholds and genotype-specific responses. Our findings reveal critical insights into optimizing chickpea production in the face of climatic challenges.

To preserve organismal and cellular homeostasis, which is also known as acclimatization, plants must continuously modify their physiology and development in response to an ever-changing biotic and abiotic environment (Sadhukhan *et al.,* 2022). Ambient temperature is one environmental characteristic that varies greatly over different time intervals. High daytime temperatures can cause several issues for cells and have a long-term negative impact on plant growth and development (Moore *et al.,* 2021). In many temperate regions, hot days and heat waves are expected to grow in frequency and intensity in the upcoming decades due to global warming (Dos Santos *et al.,* 2022). Understanding how plants respond to high temperatures is crucial for society, as humans rely entirely on agricultural production for sustenance.

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According to the reports, rising temperatures in India's dry and semi-arid regions are causing climate change, which has a negative impact on agricultural products (Shrestha *et al.,* 2023). In Northern India, chickpeas (*Cicer arietinum* L.), which are cool-season crops, are often sown in the second half of October and November. Its seeding is delayed, which eventually leads to a low seed yield, because of variations in the monsoon pattern and the late harvest of previous kharif crops like rice and sugarcane (Wang *et al.,* 2006). Legume crops are extremely sensitive to temperature variations since the late-sown crop is exposed to high temperatures, hence any shift in temperature causes major alterations in these crops (Bhandari *et al.,* 2017).

Considering the current climate change scenario brought on by global warming, meteorological parameters have a significant impact on crop productivity. In the end, this causes the nighttime temperature to drop sharply and the daytime temperature to rise. Legume crops are temperature-sensitive, but cool-season pulse crops like chickpeas (*Cicer arietinum* L.) are especially vulnerable (Raghavendra *et al.,* 2017).

Early flowering stages with low temperatures (0 to 10°C) may also result in increased floral abortion, which lowers pod and seed set (Croser *et al.,* 2003). Both the duration and rate of grain filling are sensitive to temperature changes and high temperatures may reduce productivity due to decreased flowering, pod formation and seed set, which could result in a loss of output (Srinivasan *et al.,* 1998). To mitigate the effects of high or low temperatures on chickpea productivity, it is crucial to investigate pollen traits, including pollen fertility, sterility, germination, tube length and diameter. This will allow for the identification of new chickpea varieties that are more resilient to temperature stress. The main objective of this experiment is establishing a correlation between temperature regimes and the different reproductive components in genotypes of chickpea crop.

MATERIALS AND METHODS

Location and climate

The field experiments were conducted during 2018-19 and 2019-20 *rabi* season, at the MARS, UAS, Dharwad which was located at 15012′ North latitude and 76034′ East longitude with an altitude of 678 above mean sea level. A wide range of fluctuations were observed in the mean monthly maximum and minimum temperatures during the experimental years. The maximum temperature sometimes exceeds 37°C during summer season and less than 15°C during winter season, resulted in huge amount of variation in temperature throughout the year. Since 1930, Dharwad experiences average annual rainfall of about 625.9 mm and the total rainfall recorded during 2018-19 was 891.2 mm whereas, during 2019-20 it reached up to 1316.2 mm. The values were recorded for rain fall, relative humidity and temperature throughout the experimental duration and are presented in Fig 1.

Plant materials

Six genotypes (Annigeri-1, JAKI-9218, JG-11, JG-14, BGD-128 and KAK-2) were assigned to three temperature regimes. Factorial randomized complete block design (FRCBD) was used with three replications. The experimental plots contain 14 rows of crops in 4 m × 4 m (length and width of the plots). The inter and intra-row spacings were 30 × 10 cm, respectively.

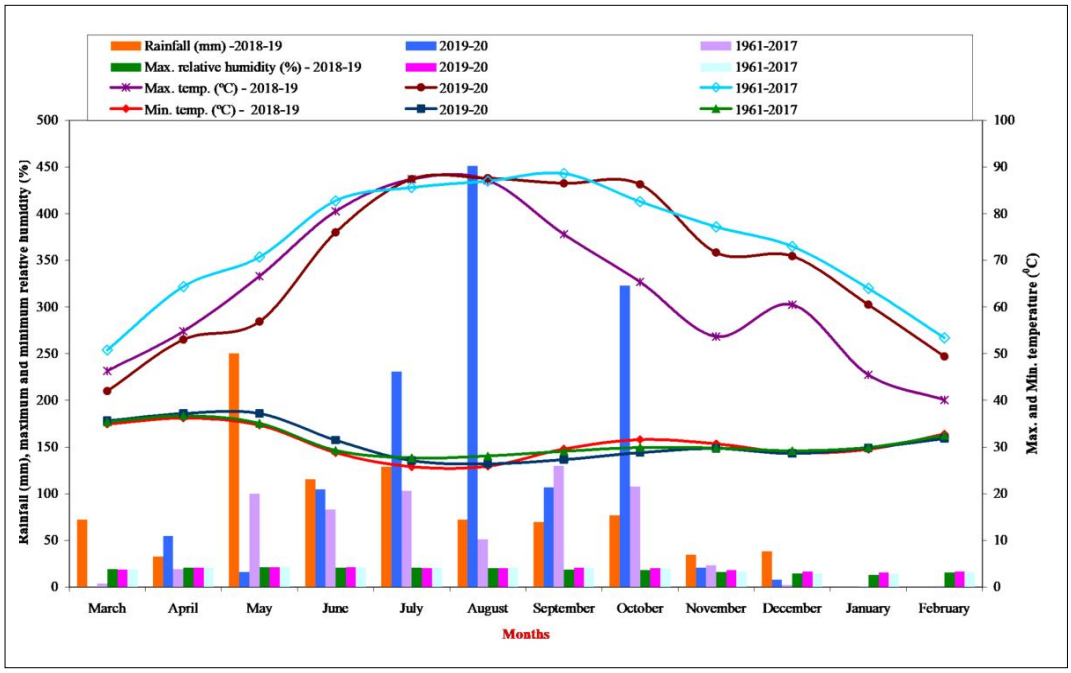


Fig 1: Monthly meteorological data at Main Agriculture Research Station (MARS), UAS, Dharwad, Karnataka.

Pollen sterility and pollen fertility

Pollen sterility and pollen fertility was observed under microscope using 1.0 per cent acetocarmine stain prepared using 1.0 g carmine, 55 ml distilled water and 45 ml acetic acid which was boiled gently for five minutes and filtered. Anthers of freshly collected flowers were dusted on slide and 20 microliters of acetocarmine solution was added to it and covered with cover slip. Stained pollen grains which are fertile and unstained grains which are sterile were counted. Then per cent pollen sterility and pollen fertility were assessed using below mentioned formula (Alexander, 1969).

$$\text{Pollen sterility (\%)} = \frac{\text{Number of sterile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Pollen germination

The collected pollen grains were incubated for 15 minutes in a prepared Kwacks media given by Brewbaker and kwack (1963) which was composed of 10% sucrose, 100 ppm boric acid (H_3BO_3), 300 ppm of calcium nitrate ($Ca(NO_3)_2$ $4H_2O$) and 200 ppm magnesium sulphate ($MgSO_4 . 7H_2O$). From this media (solution) and pollen suspension 10 µl droplets was put on the slide then the germinated pollens were recorded under compound microscope. The per cent germination was assessed using below formula:

$$\text{Pollen germination (\%)} = \frac{\text{Number of germinated pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Pollen tube length and thickness (µm)

Kwacks medium was used to access the pollen germination. The germinated pollens were observed under image analyzer microscope under 10X. Multiple views per slide were collected and mean pollen tube length and thickness was measured and expressed in µm.

Design of experiment

A factorial randomized block design (FRBD) was employed for this study, with three replicates for each treatment. ANOVA was conducted to analyze all the data and when the F test indicated significance at a p-value of 0.05, Fisher's protected least significant difference (LSD) test was used to differentiate the means (Panse and Sukhatume, 1954).

RESULTS AND DISCUSSION

Effect of sowing at different time

The correlation 'r' values for maximum, minimum temperature and temperature range for different dates of sowing had significantly strong positive correlation and negative correlation (Table 2) among reproductive traits such as pollen germination, pollen sterility, pollen fertility, fertile pollen diameter and sterile pollen diameter. The data in Table 2 clearly depicts the variation in maximum temperature minimum temperature and average temperature. Lot of variations was observed in weather data across two seasons (2018-19 and 2019-20). Among different temperature regimes maximum temperature (36.3°C) was observed due to late sowing (49th Standard Meteorological Week - 3rd December) and minimum (33.5°C) was observed due to normal sowing (45th Standard Meteorological Week - 5th November). The average temperature regime was 33.5°C during 2018-19. Whereas 34.5°C and 33.0°C were maximum and minimum temperature observed during late sowing (49th Standard Meteorological Week - 3rd December) and D1 (40th Standard Meteorological Week - 1st October) temperature regime respectively during 2019-20. Temperature range is most important aspect for reproductive traits such as pollen germination, pollen tube growth, fertilization *etc*. There was a lot of temperature range difference across two seasons and between respective temperature regimes. Between early sowing (40th Standard Meteorological Week - 1st October) of 2018-19 and 2019-20 (40th Standard Meteorological Week - 1st October) The temperature difference was 4.4°C. Between normal sowing (45th Standard Meteorological Week - 5th November) of 2018-19 and 2019-20 (45th Standard Meteorological Week - 5th November), the temperature difference was 3.2°C. Between late sowing (49th Standard Meteorological Week - 3rd December) of 2018-19 and 2019-20 (49th Standard Meteorological Week - 3rd December) the temperature difference was 5.4°C.

Table 1: The temperature variations between 2018-19 (Flowering period) and 2019-20 (Flowering period) *rabi* seasons during flowering period.

Temperature regimes	Temperature (°C)		
	Max temperature	Min temperature	Range
2018-19			
Early regime (40th SMW- 1st October)	32.10	12.20	19.90
Normal regime (45th SMW- 5th November)	31.40	9.80	21.60
Late regime (49th SMW- 3rd December)	33.40	11.70	21.70
2019-20			
Early regime (40th SMW- 1st October)	30.00	14.10	15.90
Normal regime (45th SMW- 5th November)	30.70	13.50	17.20
Late regime (49th SMW- 3rd December)	30.20	13.50	16.70

Effect on flowering and yield

There was a lot of temperature variations between flowering period and crop growth period (Table 1 and Table 2). From the earlier studies by Devasirvatham *et al.* (2012); Gaur *et al.* (2013); Krishnamurthy *et al.* (2011) it can be concluded that temperature more than 35°C during the reproductive phase was considered very crucial for growth, development and optimum yield. Further, the pod set of chickpea genotypes was reduced by 50 per cent at 35°C. Reproductive *via*bility should be maintained and it could be used as a *via*ble tolerance mechanism under high temperature stress conditions. A similar report of yield reduction in heat tolerant varieties under high temperature was also reported in wheat (Barber *et al.,* 2017; Tang *et al.,* 2018) and peas (Tafesse *et al.,* 2019).

Effect on pollen characteristics

Pollen viability, *in-vitro* pollen germination and *in vitro* pollen tube growth were significantly declined under high temperature stress in all the genotypes. This indicated that the tolerant genotypes could defend against the damages that are caused by the high temperature on growth of male reproductive organs with the help of various defense mechanisms, that are induced under such unfavorable conditions (Kumar *et al.,* 2013; Saini *et al.,* 2022).

Variation in day/night temperature (diurnal variation) *viz.,* high day (33.40/30.00°C) and low night (18.9/9.80°C) temperature reduced the pollen germination, pollen tube length, pollen fertility and pollen diameter. This study demonstrated that the temperature stress affected the pollen morphology of tolerant and susceptible chickpea genotypes as evident varying pollen germination and pollen *via*bility when exposed to high/low temperature. Similar temperature effects on pollen germination were also reported by (20). The modification in pollen morphology may be associated with the reduced accumulation of starch in microspores. Similar observation was also recorded in wheat, starch requirement for pollen germination varies with the pollen development phases, the peak requirement will be in bicellular stage (Sadhukhan *et al.,* 2022). A reduction in the starch due to low/high temperature stress during this stage causes disrupted pollen growth. However, the genotypes BGD-128, JAKI-9218, JG-11 and JG-14 have overcome this phenomenon through breakdown of triacylglycerols for the energy source instead of carbohydrates. Similar report of using triacylglycerols under stress condition as an energy source was also reported (Moore *et al.,* 2021).

Table 3 depicts the overall correlation between different reproductive components of chickpea genotypes. In Annigeri-1 genotype, during 2018-19 positive correlation was observed between maximum temperature and fertile pollen diameter (0.730**). At pooled condition, positive correlation was observed between maximum temperature and pollen tube length (0.488**). In JAKI-9218 genotype, positive correlation was observed for maximum temperature with pollen tube length and sterile pollen diameter during 2018-19 (0.770**, 0.545*) and 2019-20 (0.548*, 0.542*) and the average of (pooled) of two years (0.543**, 0.633**), respectively. Similarly the average of two years the maximum temperature was positively correlated with pollen germination (0.381*) and fertile pollen diameter (0.437*) similarly, the temperature range was positively correlated with sterile pollen diameter (0.483**) at pooled condition. In the present investigation it was found that the temperature difference between 40th Standard Meteorological Week early sowing to 45th Standard Meteorological Week normal sowing 3.2°C, 49th Standard Meteorological Week late sowing to 40th Standard Meteorological Week early sowing was 5.12°C and 49th Standard Meteorological Week (D_3) to 45th Standard Meteorological Week (D_2) was 1.92°C across two seasons during crop growth period. Temperature difference between early sowing of during 2018-19 and 2019-20 was 2.56°C, between normal sowing of 2018-19 to 2019-20 was 2.77°C and between late sowing of 2018-19 to 2019-20 was 2.38°C.

Maximum temperature was positively correlated with pollen germination (0.389*) in JG-11 genotype at pooled condition. Average two years seasons during 2019-20 in JG-14 genotype, maximum temperature was positively correlated with pollen germination (0.622*) and pollen tube length (0.644**), similarly, the temperature range was positively correlated with pollen tube length (0.536*). The average of two seasons the maximum temperature was positively correlated with pollen fertility (0.375*), whereas,

Table 2: Meteorological data recorded during crop growth period of *rabi* 2018-19 and 2019-20.

Temperature regimes	Temperature (°C)			Relative humidity (%)			Rain fall (mm)
	Min	Max	Range	Min	Max	Range	
2018-19							
Early regime (40th SMW- 1st October)	9.0	33.8	24.8	17.0	96.0	79	135.8
Normal regime (45th SMW- 5th November)	9.0	33.5	24.5	17.0	96.0	79	72.8
Late regime (49th SMW- 3rd December)	9.0	36.3	27.3	11.0	94.0	83	0.0
2019-20							
Early regime (40th SMW- 1st October)	12.6	33.0	20.4	23.0	98.0	75	332.8
Normal regime (45th SMW- 5th November)	12.6	33.9	21.3	23.0	98.0	75	7.8
Late regime (49th SMW- 3rd December)	12.6	34.5	21.9	18.0	98.0	80	0.0

negatively correlated with pollen sterility (-0.375*). The pooled average of two seasons the maximum temperature was positively correlated with pollen fertility (0.506**) in KAK-2 genotype, whereas, negatively correlated with pollen sterility (-0.*506**) (Plate 1) With respect to BGD-128 genotype, there was no correlation between maximum, minimum and temperature range reproductive traits such as pollen germination, pollen sterility, pollen fertility, fertile pollen tube length and sterile pollen tube length across two seasons 2018-19 and 2019-20. The results of the

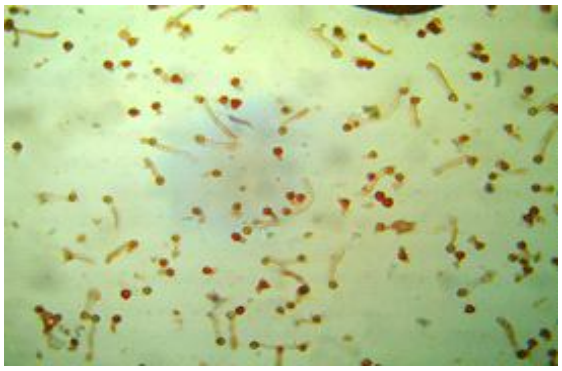
Table 3: Correlation studies of Tmax, Tmin and Trange temperature on reproductive traits of chickpea genotypes.

	2018-19			2019-20			Pooled		
	T max	Tmin	T range	T max	Tmin	T range	T max	Tmin	T range
Annigeri-1									
PG	0.064	-0.116	0.140	0.383	0.449	-0.035	0.170	0.160	-0.046
PS	0.159	-0.040	0.096	0.007	-0.159	0.110	-0.140	0.026	-0.092
PF	-0.159	0.040	-0.096	-0.007	0.159	-0.110	0.140	-0.026	0.092
PT	0.038	0.086	-0.074	0.428	0.234	0.138	0.488**	-0.060	0.299
FPD	0.730**	0.119	0.135	-0.182	0.462	-0.431	0.308	0.149	0.033
SPD	0.235	-0.223	0.310	0.198	0.137	0.045	-0.116	0.047	-0.098
JAKI-9218									
PG	0.796**	0.064	0.231	0.223	0.322	-0.063	0.381*	0.098	0.114
PS	0.136	-0.093	0.143	-0.073	0.191	-0.181	-0.080	0.071	-0.099
PF	-0.136	0.093	-0.143	-0.006	0.252	-0.175	0.096	0.047	0.011
PT	0.770**	0.263	0.023	0.548*	0.478	0.058	0.543**	0.313	0.021
FPD	0.025	-0.013	0.022	0.403	0.181	0.158	0.437*	-0.103	0.308
SPD	0.545*	-0.140	0.341	0.542*	-0.017	0.389	0.633**	-0.195	0.483**
JG-11									
PG	0.319	0.182	-0.074	0.459	0.445	-0.007	0.389*	0.233	0.004
PS	-0.077	-0.016	-0.011	-0.109	-0.446	0.235	-0.220	-0.087	-0.040
PF	-0.272	-0.061	-0.033	0.109	0.446	-0.235	-0.226	0.029	-0.139
PT	0.365	-0.115	0.245	0.344	0.336	-0.007	0.374	-0.020	0.208
FPD	-0.080	-0.211	0.187	0.181	0.483	-0.214	0.092	0.007	0.041
SPD	-0.289	-0.331	0.237	0.053	0.188	-0.095	0.146	-0.149	0.198
JG-14									
PG	0.029	-0.140	0.152	0.622*	0.185	0.307	0.121	-0.048	0.103
PS	-0.238	0.298	-0.393	-0.467	-0.280	-0.136	-0.375*	0.160	-0.329
PF	0.238	-0.298	0.393	0.467	0.280	0.136	0.375*	-0.160	0.329
PT	0.270	0.192	-0.088	0.644**	-0.128	0.536*	0.194	0.188	-0.053
FPD	-0.166	-0.194	0.131	0.273	0.279	0.001	0.027	-0.167	0.152
SPD	-0.135	0.070	-0.122	0.472	0.171	0.213	-0.119	0.166	-0.199
BGD-128									
PG	-0.064	0.013	-0.034	0.441	0.132	0.191	-0.031	0.109	-0.109
PS	0.004	0.163	-0.173	-0.070	-0.480	0.285	-0.046	-0.008	-0.017
PF	-0.020	0.126	-0.142	0.070	0.480	-0.285	-0.085	0.192	-0.208
PT	0.134	-0.100	0.150	-0.096	0.279	-0.253	-0.177	0.083	-0.161
FPD	-0.009	-0.033	0.032	0.399	0.195	0.122	0.314	0.004	0.155
SPD	0.309	-0.161	0.271	0.127	0.496	-0.259	-0.217	0.244	-0.320
KAK-2									
G	0.381	-0.020	0.142	0.326	0.274	0.024	0.142	0.100	-0.014
PS	-0.365	0.015	-0.132	-0.312	-0.203	-0.063	0.506**	0.065	-0.306
PF	0.365	-0.015	0.132	0.312	0.203	0.063	0.506**	-0.065	0.306
PT	0.410	0.025	0.105	0.468	0.414	0.020	0.387*	0.123	0.089
FPD	0.248	0.232	-0.161	-0.051	0.437	-0.332	-0.032	0.301	-0.271
SPD	0.135	-0.075	0.121	-0.022	0.390	-0.282	-0.225	0.175	-0.260

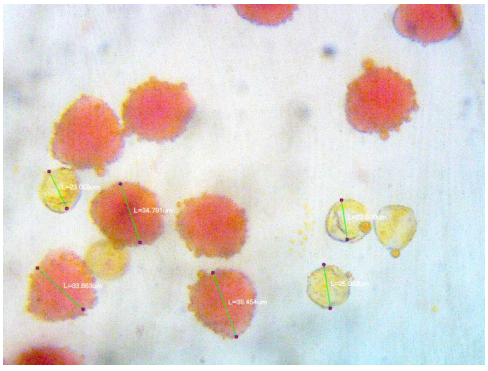
Note: T max- Temperature maximum, T min- Temperature minimum, T range- Temperature range.

PG: Pollen germination, PF: Pollen fertility, PS: Pollen sterility.

PT: Pollen tube length, FPD: Fertile pollen diameter, SPD: Sterile pollen diameter.



a) Pollen germination



b) Pollen viability and pollen sterility

Plate 1: Pollen germination, Fertile and sterile pollen diameters under 100 X.

studies indicated that maximum temperature was positively correlated with all reproductive components except pollen sterility whereas, there was no correlation exited with respect to minimum temperature, but temperature range is positively correlated with sterile pollen diameter in JAKI-9218 genotype. Like in JG-14 genotype, positive correlation was found with respect to pollen tube length.

The findings show that chickpea genotypes' ability to reproduce is significantly influenced by temperature. Better reproductive outcomes are supported by optimal temperatures; departures from this range, particularly at extreme temperatures, result in reduced flowering, pod set and seed yield. Genotypes vary in their response to temperature stress, with Annigeri-1 and KAK-2 being the most adversely affected. These findings align with recent research highlighting genetic variability in chickpea's heat tolerance (Kumar *et al.,* 2013; Saini *et al.,* 2022; Jeffrey *et al.,* 2021).

Similar findings of improved pollen tube length, germination and fertility was also positively correlated in tomato (Pham *et al.,* 2020), hybrid shrub roses (Bheemanahalli *et al.,* 2021) and rice. Generally, under high temperatures, ROS will be overproduced causing a decline in pollen germination, while the tolerant cultivars may have the overriding mechanism through synthesis of antioxidants *i.e.* ascorbate peroxidase (APX) and superoxide dismutase (SOD) (Singh *et al.,* 2024). The study underlines the necessity of developing heat-resilient chickpea cultivars to provide stable output under changing climatic circumstances. Future study should focus on clarifying the genetic and physiological factors causing heat tolerance in chickpeas and incorporating these qualities into breeding strategies.

CONCLUSION

From the outcome of the investigation, it was concluded that the characteristics such as pollen germination, pollen tube length, pollen sterility, pollen fertility percentage, pollen fertile diameter and sterile pollen diameter were strongly influenced by temperature. The high temperature stress affected the pollen morphology of tolerant and susceptible chickpea genotypes as evident by varying pollen germination and pollen *via*bility. The chickpea genotypes such as BGD-128, JAKI-9218, JG-11 and JG-14 could be used in the breeding program for selection of heat tolerant genotypes for higher economic chickpea yield. Temperature regimes have a substantial effect on chickpea genotypes' ability to reproduce, with various genotypes exhibiting differing degrees of sensitivity. Comprehending these reactions is crucial for cultivating heat-tolerant cultivars and reducing the effects of global warming on the production of chickpeas.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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