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Research Article *Natural Resource Management*

Effect of Heat Stress on Flower Anatomy of Heat Tolerance and Susceptible Chickpea (*Cicer arietinum* L.) Genotypes by Scanning Electron Microscopy

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Abstract

With increasing globally averaged temperature of the air above the earth's surface would raise by 1.4- 5.8 °C over the next 100 years and its negative impact on reproductive stage is thus increasingly becoming a serious constraint to chickpea production in India. The present study was undertaken to exogenous application of salicylic acid at flowering stage in four chickpea genotypes (ICC-4958, GG-2, ICC-4567 and GJG-5) under normal sowing (14th November, 2018) and late sowing (12th December, 2018). Chickpea has a strong indeterminate growth habit, and generally it produces many flowers, but only 50% to 80% of these develop into mature pods due to the failure of pod set and pod abortion. A few days of exposure to high temperatures (30-35°C) causes heavy yield losses through accelerating flower drop and pod abortion. Heat stress inhibited pollen function and pollen tube growth more in the heat susceptible genotypes (ICC-4567 and GJG-5) than in the tolerant genotypes (ICC-4958 and GG-2). The exogenous application of SA at 50% flowering stage in late sowing showed that the significant difference was found in number of flowers per plant and there was no significant difference found in number of flowers per plant during normal sowing. In all genotypes number of flower plant^{-1} increased after spraying 100 ppm SA as compared to control. Heat stress inhibited pollen function and pollen tube growth more in the sensitive genotypes than in the tolerant genotypes.

Keywords: Salicylic acid, heat stress, pollen size, style length

1. Introduction

Chickpea is the third most important crop representing about 30% of the land area under pulse, which contributes 40% of the total pulse production in india. In India, chickpea is grown over an area of 10.56 mha with production of 11.379 mt and average productivity of 1078 kg ha^{-1} (Anonymous, 2018). Chickpea (*Cicer arietinum* L.) is cultivated in arid and semi-arid areas around the world. Chickpea belongs to genus *Cicer*, tribe *cicerace*, family sp. *Fabaceae* and subfamily *Faboideae.* Chickpea popularly known as Gram, Bengal gram, Homes, Chhola, Garbanzo bean is one of the first seed legumes to be domesticated by humans in old world. The origin of the crop is considered to be Western Asia from where it spread in India and other parts of the world (Rathore and Sharma, 2003). The Inter Governmental Panel on Climate Change (IPCC) of the United Nations in its recent report has projected that the globally average temperature of the air above the earth's surface would rise by 1.4-5.8 °C over the next 100 years (IPCC, 2019). Recently the IPCC

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has updated that the earth temperature has increased by 0.75 °C to 0.99 °C between 1906 and 2018 due to increase in anthropogenic emission of green house gases. For Indian regions (south Asia), the IPCC has projected 0.5-1.2 °C rise in temperature by 2020, 0.88-3.16 °C by 2050 and 1.56-5.44 °C by 2080 depending on the scenario of future development (IPCC, 2007). Global estimates of chickpea yield loss because of drought and heat stress have indicated average yearly losses of 3.3 mt. These abiotic stresses lead to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity of chickpea (Basu et al., 2008).

Chickpea has a strong indeterminate growth habit, and generally it produces many flowers, but only 50% to 80% of these develop into mature pods due to the failure of pod set and pod abortion. A few days of exposure to high temperatures (30-35 °C) causes heavy yield losses through accelerating flower drop and pod abortion. In general heat stress has negative effects on production, especially during gamete development, flowering and podding. Yield reductions have been observed at a temperature of 30 °C at 50% flowering and >30 °C for 3-4 days at 100% flowering (Summerfield et al., 1984; Wang et al., 2006, Devasirvatham et al., 2012). Low temperature, at initial stage of crop growth results in poor and slow vegetative growth whereas, high temperature at the end of cropping season leads to forced maturity and poor biomass in chickpea (Clarke and Siddique, 2004). Temperature stress at reproductive stage is thus increasingly becoming a serious constraint to chickpea production in India due to climate change. High temperature adversely affects seed germination, photosynthesis, respiration, membrane stability, fertilization, fruit maturation, quality of seeds, nutrient absorption, protoplasmic movement, transport of materials and also modulated level of hormones and primary and secondary metabolites. It leads to the changes of a large number of genes in plants (Kamal et al., 2010). Expose of the heat stress in chickpea during flowering and podding stage to very sensitive in external environment and exposure to heat stress at this stage leads to reduction in seed yield (Kaushal et al., 2013).

Plants have evolved some remarkable chemical substances, often to defend themselves against being eaten. Among various phenolic substances, particularly salicylic acid exerts its influences on plant growth and development, photosynthetic machinery, flowering, membrane permeability, and enzyme activities (Yusuf et al., 2013). Growth and development of plants, like all organisms, is regulated by various internal external stimuli. In recent years, SA has been in focus of intensive research due to its crucial role in the regulation of physiological and biochemical processes during the entire life span of the plants and plays key roles in regulating their growth and productivity (Arberg, 1981).

2. Materials and Methods

2.1. Plant material

Heat tolerant (ICC-4958 and GG-2) and susceptible (ICC-4567 and GJG-5) genotypes were procured from ICRISAT, Hyderabad and Pulse Research Station, J.A.U., Junagadh. An experiment was conducted at two different dates normal sowing (14th November, 2018 to 28th February, 2019) and late sowing (12th December, 2018 to 31st December, 2019) in field condition during 2018-2019 *rabi*. Spraying of salicylic acid in different concentration (T_1: Control, T_2: 100 ppm, T_3: 200 ppm and T_4: 400 ppm) at 50% flowering stage and further sample was collected after two days for scanning electron microscopy analysis. Total number of flower plant^{-1} was being counted at the time of 50% flowering stage.

2.2. Scanning electron microscopy (SEM)

SEM of flower was done by standard method to observe pollen size and style length (Talbot and White, 2013; Reynolds et al., 1994). Samples were fixed in 3% glutaraldehyde buffered with 0.1 M phosphate buffer at room temperature for 2 hr. Sample were washed with 0.1 M phosphate buffer and immerse in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) for 2h at room temperature and in a light proof container. Samples were again washed in 0.1 M phosphate buffer (pH 7.2), dehydrated in a graded ethanol solutions prepared in distilled water (30%, 50%, 70% 80%, 90%, 96%, and 100%) for 5-15 min. and allowed it to dry. This complicated process involves simply the replacement of liquid in the cells with gas. This process creates a completely dry specimen with minimal or no cellular distortion. Mount the specimen on an aluminium stub using double sided sticky carbon tape. Gold coating was performed using gold sputter coater that coats the mounted specimens in gold before they go into the SEM. Then samples were analysed directly in SEM using Smart SEM TM software (Carl Zeiss, EVO18, UK) at Junagadh Agricultural University, Junagadh; Gujarat.

3. Results and Discussion

Heat tolerant and susceptible genotypes of chickpea grown under two different sowing dates normal sowing and late sowing. Different concentration of salicylic acid spray at 50% flowering stage in both normal and late sowing. In this treatment there was significant effect on number of flower per plant (Table 1) and pollen length and width (Figure 1A, B, C and D).

The characterization of pollen and style morphology was carried out using SEM. The results of analysis of variance was indicated significant differences in pollen length/width and style length among heat tolerant and susceptible genotypes.

In normal sowing number of flowers per plant in heat tolerant and susceptible chickpea genotypes ranged from 126.50 to 132.00. The genotype ICC-4567 (132.00) showed the maximum numbers of flowers per plant followed by GG-2 (129.17). The genotype GJG-5 (126.50) had the minimum numbers of flowers per plant followed by ICC-4958 (127.08).

Table 1: Effect of salicylic acid on number of flower per plant in chickpea at 50% flowering stage in different sowing date

Normal sowing					
Genotype (G)	Treatment (T)				Mean (G)
	T_1	T_2	T_3	T_4	
ICC-4958 (T)	129.33	131.33	124.67	123.00	127.08
ICC-4567 (S)	128.00	128.67	138.67	132.67	132.00
GG-2 (T)	128.67	130.67	122.67	134.67	129.17
GJG-5 (S)	128.00	126.67	126.67	124.67	126.50
Mean (T)	128.50	129.33	128.17	128.75	
	SEm±	CD(p=0.05)		CV%	
G	1.56	NS		4.21	
T	1.56	NS			
G X T	3.13	9.03			
Late Sowing					
Genotype (G)	Treatment (T)				Mean (G)
	T_1	T_2	T_3	T_4	
ICC-4958 (T)	121.93	125.93	123.67	119.13	122.67
ICC-4567 (S)	124.8	126.20	127.40	128.40	126.70
GG-2 (T)	125.27	126.60	124.93	127.53	126.08
GJG-5 (S)	120.4	127.33	124.87	123.80	124.10
Mean (T)	123.10	126.52	125.22	124.72	
	SEm±	CD(p=0.05)		CV%	
G	0.68	1.96		1.88	
T	0.68	1.96			
G X T	1.36	3.92			

The highest number of flowers plant^{-1} found in treatment T_2 (129.33) followed by T_4 (128.75). The treatment T_3 (128.17) had minimum number of flowers plant^{-1} followed by T_1 (128.5). The combine effect of treatments and genotypes found to be significant in normal sowing for number of flowers per plant (Table 1). In all genotypes number of flowers per plant increased after spraying of 100 ppm SA (T_2) as compared to control (T_1) except genotype GJG-5.

In late sowing number of flowers plant^{-1} in heat tolerant and susceptible chickpea genotypes ranged from 122.67 to 126.70. The genotype ICC-4567 (126.67) showed the maximum numbers of flowers plant^{-1} followed by GG-2 (126.08). The genotype ICC-4958 (122.70) had the minimum numbers of flowers plant^{-1} followed by GJG-5 (124.10). The highest number of flowers plant^{-1} found in treatment T_2 (126.52) followed by T_3 (125.22). The treatment T_1 (123.10) had minimum number of flowers plant^{-1} followed by T_4 (124.72).

The combine effect of treatments and genotypes found to be significant in late sowing for number of flowers per plant (Table 1). In all genotypes number of flowers plant^{-1} increased after spraying of 100 ppm SA (T_2) as compared to control (T_1).

Pancheya et al. (1996) found that high concentration of SA causes reduction of photosynthetic rate and Rubisco activity in plant. Plants have evolved some remarkable chemical substances, often to defend themselves against being eaten. Among various phenolic substances, particularly SA exerts its influences on plant growth and development, photosynthetic machinery, flowering, membrane permeability, and enzyme activities (Arberg, 1981).

In T_1 treatment pollen length of tolerant genotype (ICC-4958) decreased in late sowing (33.51 µm) as compared to normal sowing (35.06 µm). Whereas, pollen width increased in late sowing (20.97 µm) compared to normal sowing (19.82 µm). In same genotype pollen length increases in T_2 and T_4 treatment in late sowing (35.92 µm and 34.10 µm) compared to normal sowing (34.04 µm and 33.92 µm). However, pollen width increased in T_2 and T_3 treatment in late sowing (18.63 µm and 20.42 µm). Whereas, in T_4 treatment pollen width declined in late sowing (18.60 µm) compared to normal sowing (19.04 µm).

In susceptible genotype (ICC-4567) pollen length decline in late sowing (23.44 µm, 34.20 µm and 33.38 µm) compare

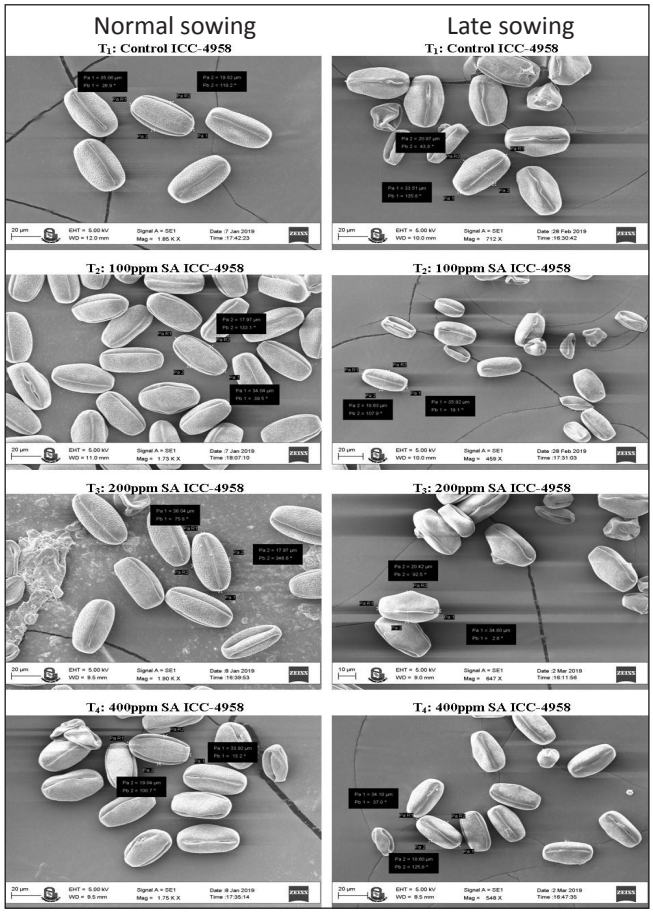

Figure 1A: Effect of salicylic acid on pollen length and width in heat tolerant (ICC-4958) genotype under different sowing dates

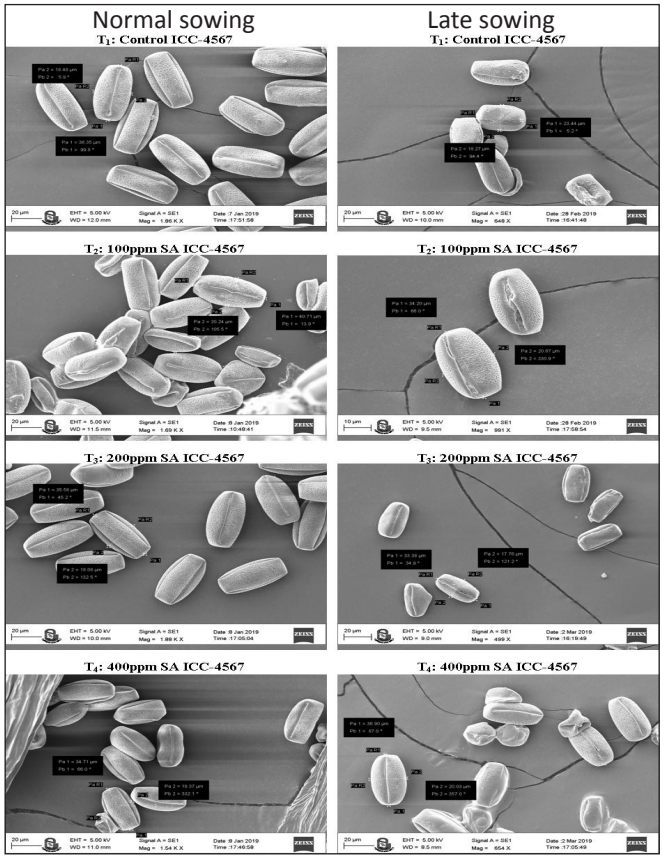

Figure 1B: Effect of salicylic acid on pollen length and width in heat susceptible (ICC-4567) genotype under different sowing dates

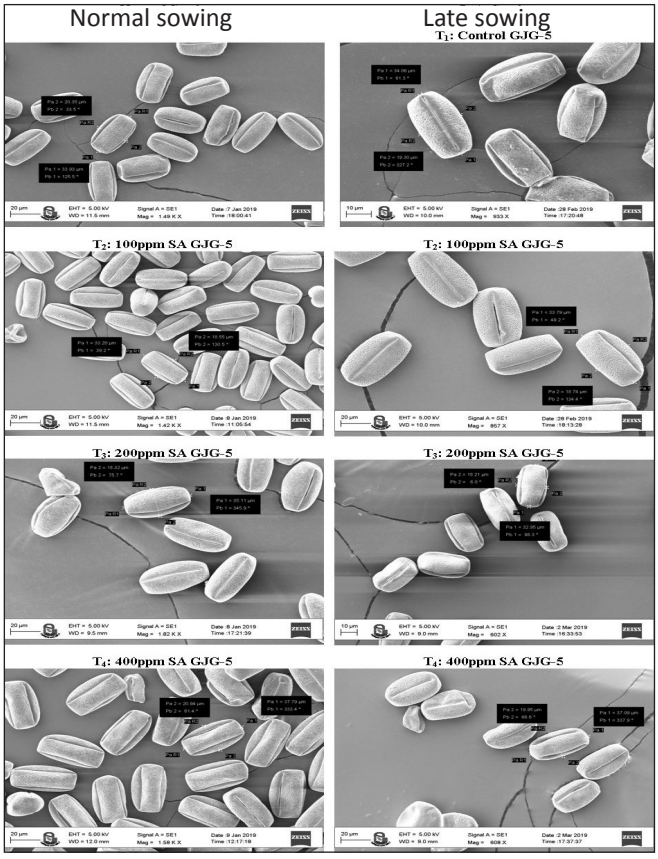

Figure 1D: Effect of salicylic acid on pollen length and width in heat susceptible (GJG-5) genotype under different sowing dates

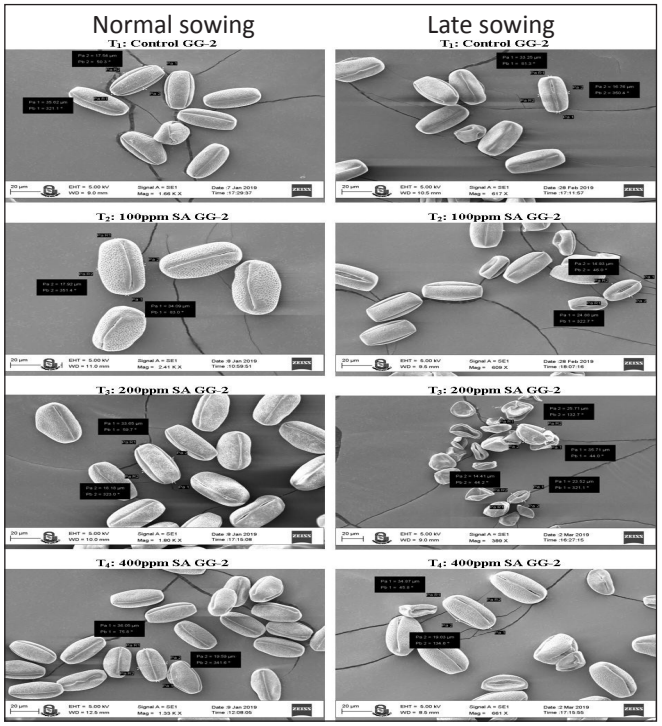

Figure 1C: Effect of salicylic acid on pollen length and width in heat tolerant (GG-2) genotype under different sowing dates

to normal sowing (36.35 µm, 40.71 µm and 35.58 µm) in T_1, T_2 and T_3 treatments. However, treatment T_4 pollen length increased in late sowing (36.90 µm) compare to normal sowing (34.71 µm). Moreover, pollen width decline in T_1 and T_3 treatments in late sowing (18.27 µm and 17.76 µm) compare to normal sowing (18.48 µm and 18.08 µm). Whereas, pollen width increased in late sowing (20.67 µm and 20.03 µm) compare to normal sowing (20.24 µm and 18.37 µm) in T_2 and T_4 treatments.

In tolerant genotype (GG-2) pollen length decline in late sowing (33.25 µm, 24.88 µm, 23.52 µm and 34.87 µm) as compare to normal (35.62 µm, 34.09 µm, 35.65 µm and 36.05 µm) sowing in all treatments. However, pollen width also decline in late sowing (16.76 µm, 14.03 µm, 14.41 µm and 19.03 µm) as compare to normal sowing (17.54 µm, 17.92 µm, 18.18 µm and 19.59 µm) in all treatments.

When susceptible genotype (GJG-5) expose to heat stress during late sowing (34.06 µm and 33.79 µm) condition pollen length increase compare to normal sowing (33.93 µm and 33.20 µm) in T_1 and T_2. However, in T_3 and T_4 treatments pollen length decreased in late sowing (32.95 µm and 37.09 µm) as compare to normal (35.11 µm and 37.79 µm) sowing. In addition, pollen width decreased in all treatments when susceptible genotype expose to heat stress during late sowing

condition except T_2.

Effect of different concentration of salicylic acid spray on style length at 50% flowering stage were also studied in both normal and late sowing. In these treatments there was no any significant difference was observed on style length (Figure 2A, B, C and D). The style length of tolerant genotype (ICC-4958) decreased in late sowing (2.89 µm, 3.02 µm, 2.60 µm and 3.05 µm) as compared to normal sowing (3.59 µm, 3.08 µm, 3.37 µm and 3.12 µm) in T_1, T_2, T_3 and T_4 treatment. In susceptible genotype (ICC-4567) style length decline in late sowing (2.59 µm) as compared to normal sowing (3.00 µm) in T_4 treatment. However, in treatment T_1, T_2 and T_3 style length increased in late sowing (2.95 µm, 3.43 µm and 2.38 µm) as compared to normal sowing (2.60 µm, 2.76 µm and 3.23 µm). The style length of tolerant genotype (GG-2) decline in late sowing (3.38 µm, 2.91 µm, 2.62 µm and 3.61 µm) as compare to normal sowing (3.70 µm, 3.85 µm, 3.50 µm and 3.66 µm) in T_1, T_2, T_3 and T_4 treatment.

When susceptible genotype (GJG-5) exposed to heat stress during late sowing (3.37 µm), style length increased as compared to normal sowing (2.80 µm) in T_3 treatment. However, in T_1, T_2 and T_4 treatments style length decreased

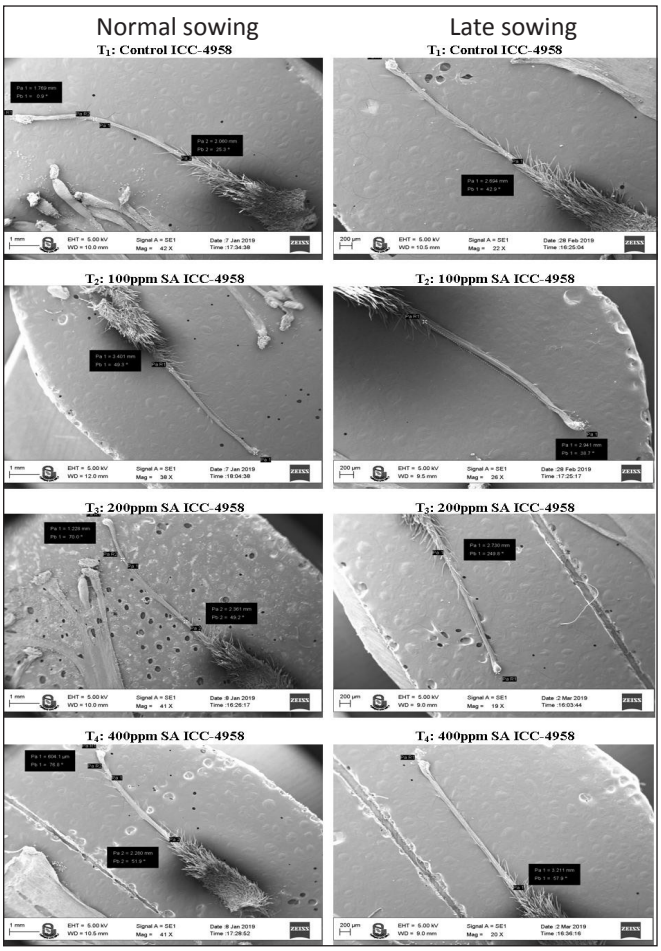

Figure 2A: Effect of salicylic acid on style length in heat tolerant (ICC-4958) genotype under different sowing dates

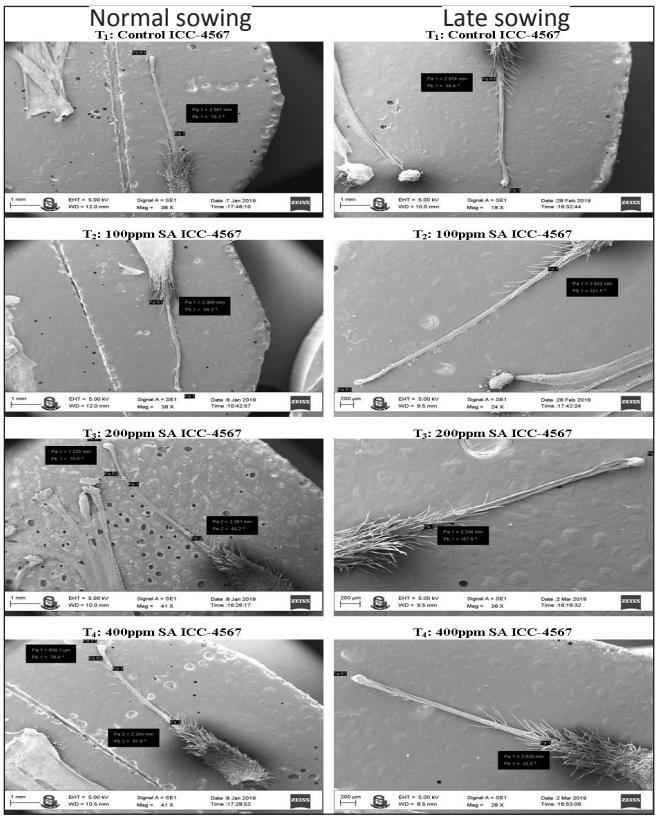

Figure 2B: Effect of salicylic acid on style length in heat susceptible (ICC-4567) genotype under different sowing dates

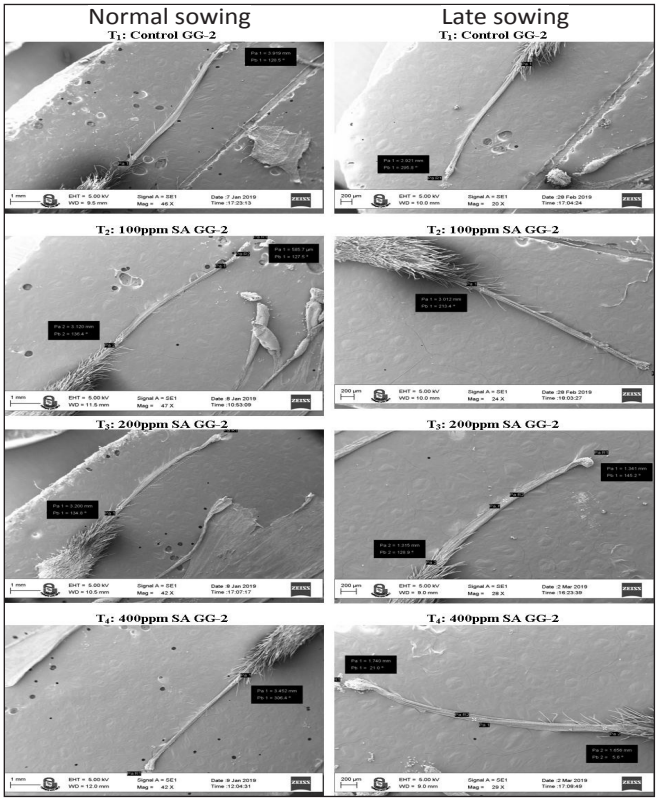

Figure 2C: Effect of salicylic acid on style length in heat tolerant (GG-2) genotype under different sowing dates

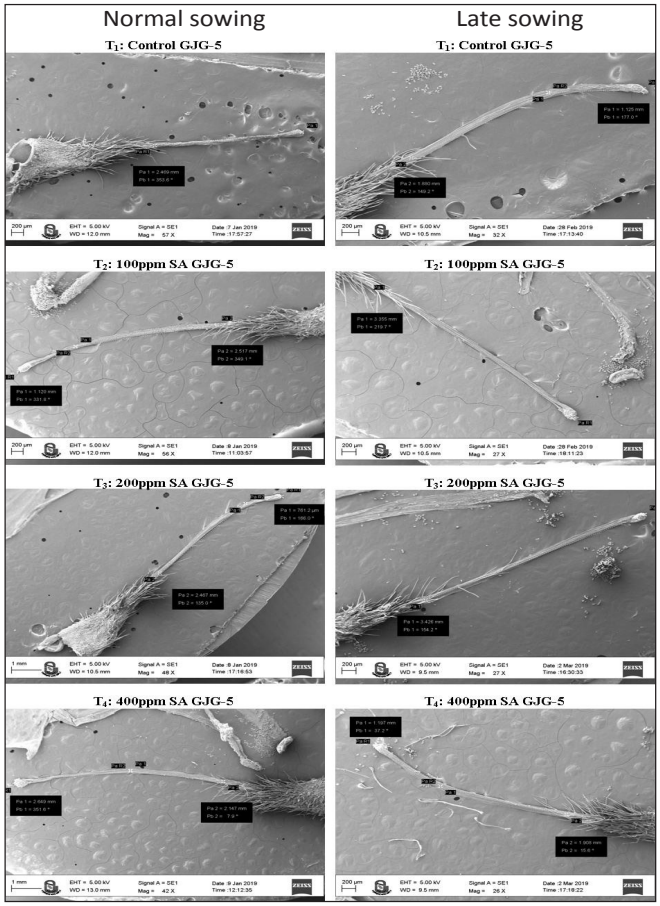

Figure 2D: Effect of salicylic acid on style length in heat susceptible (GJG-5) variety under different sowing dates

in late sowing (2.70 µm, 3.35 µm and 3.39 µm) as compared to normal sowing (2.71 µm, 3.52 µm and 3.89 µm).

Our results match with the heat stress reduced pollen size and style length. Although no visible morphological differences were observed on the surface of the pollen between the normal sowing and late sowing, the pollen length decreased due to heat stress (Late sowing) in all genotypes and width increase due to late sowing as compared to normal sowing. Hinojosa et al. (2018) evaluate quinoa growth and pollen morphology in response to high temperatures. Pollen morphology and viability and several physiological parameters were measured at anthesis in two genotypes of quinoa subjected to day/night temperatures of 22/16 °C as a control treatment and 40/24 °C as the heat stress treatment. Results showed that heat stress reduced the pollen size.

4. Conclusion

We have concluded to high temperature 34 °C negatively affects pollen size, style length and pollen tube growth thus reducing subsequent pod set. Therefore, pre-anthesis and anthesis are the most sensitive stages to high temperature in chickpea. Among the four genotypes tested, ICC-4958 and GG-2 was the most heat tolerant genotype and ICC-4567 and GJG-5 the most heat sensitive. Application of SA no significant difference in pollen size but significantly in number of flower plant^{-1}.

5. Acknowledgment

Authors are thankful to the Department of Biotechnology, Junagadh Agricultural University, Junagadh for providing necessary facilities to conduct this experiment.

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