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Effect of higher temperature on leaf anatomy of heat tolerance and heat susceptible wheat genotypes (*Triticum aestivum* L.) by scanning electron microscopy

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Abstract
A heat tolerance (DBW-150, GW-463, WH-1179, J-2015-03, HD-3118) and heat susceptible (WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725) wheat genotypes were selected and grown up to tillering and grain filling stage. The plants were subjected to two different dates: 1st sowing was done at the date of 28th November 2016 and 2nd sowing was done at the date of 31st December 2016. Scanning electron microscopy of wheat leaves showed differences for stomata width among heat tolerance and susceptible genotypes. Also anatomy was affected adversely under higher temperature in all ten genotypes leaf but with more affected in susceptible as compared to tolerance genotypes.

Keywords: Heat tolerance, Heat susceptible, Wheat, SEM

Introduction
Wheat is a food of millions of people and forms an important part of many people's daily diet and mainly utilized as flour for the production of products such as different types of bread, cakes and other baked products (Curtis *et al.*, 2002) [4]. Wheat is self-pollinated crop. It is most successfully grown between the latitudes of 30° and 60°N and 27° and 40° S (Nuttonson, 1955) [8], but it can be grown beyond these latitudes limits, from within the Arctic Circle to higher elevation near the equator. The optimum temperature for wheat growth is 25°C with minimum and maximum growth temperatures of 3°C to 4°C and 30°C to 32°C, respectively (Briggle, 1980) [3]. Wheat is being harvested in the world at different times of the year. Heat stress affects the morphological traits in addition to physiological and biochemical responses within plant cell. The yield and quality of wheat are severely affected by heat stress in many countries. Wheat genotypes express a differential response to chronic heat as well as a heat shock. Temperature requirements and temperature extremes varies widely for different cultivars of the same or different species. The reproductive phase of many crop species is relatively more sensitive to heat stress than vegetative phase (Hall, 1992) [5] (Martiniello and Teixeira, 2011) [6]. The total area of wheat production affected by some form of heat stress is estimated to be 65 to 70 million ha. Of these, 7 million ha are grown under continual heat stress (Reynolds *et al*., 1994) [10]. In present investigation effect of high temperature on 10 wheat genotypes *viz.,* Heat tolerance (DBW-150, GW-463, WH-1179, J-2015-03, HD-3118) and heat susceptible (WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725) were studied.

Material and Methods
Plant growth condition, sampling description and heat treatments: Five heat tolerance DBW-150, GW-463, WH-1179, J-2015-03, HD-3118 and five heat susceptible WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725 wheat genotypes were selected on the basis of Heat Susceptibility Index (HSI) was 0.35, 0.48, 0.50, 0.38, 1.1, 1.2, 1.1, 1.1, 1.3, respectively except J-15-03 (Anon. 2015) [1]. They were grown in natural field condition into two different showing dates. Normal sowing was done on 28th November 2016 and late sowing was done on 31st December 2016. The samples of both types of genotypes were collected and immediately analysed under scanning electron microscope.
Anatomical study: Scanning electron microscopy of stomata was done by standard method (Talbot and White, 2013) [11] (Reynolds *et. al.,* 1994) [10]. 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), dehydrate in a graded ethanol solutions in water– 30%, 50%, 70% 80%, 90%, 96%, and 100% for 5-15 min. and dried.

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 Agriculture University, Junagadh, Gujarat.

Result

In 1^{st} date stomata length was recorded 21.36 µm, 10.56 µm, 32.95 µm, 40.78 µm, and 29.20 µm with an average of 26.97 µm for heat tolerance genotype DBW-150, GW-463, WH-1179, J-2015-03, and HD-3118. In heat susceptible genotypes WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725 the length was recorded 29.64 µm, 23.57 µm, 40.66 µm, 37.59 µm and 27.41 µm, respectively, with an average of 31.77 µm.

The width of stomata was recorded in 1^{st} date 1.286 µm, 432.8 nm, 1.324 µm, 1.351 µm and 1.814 µm with an average of 1.242 µm for heat tolerance genotype DBW-150, GW-463, WH-1179, J-2015-03, and HD-3118, respectively. In heat susceptible genotype WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725 the width was recorded 1.045 µm, 1.065 µm, 2.160 µm, 1.085 µm and 1.458 µm, respectively, with the average of 1362 nm.

In 2^{nd} date stomata length was recorded 18.23 µm, 27.49 µm, 16.23 µm, 28.72 µm, and 29.01 µm, with an average 23.94 µm. for heat tolerance genotype DBW-150, GW-463, WH-1179, J-2015-03, and HD-3118, respectively. In heat susceptible genotype WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725 the length was recorded 34.98 µm, 41.89 µm, 35.89 µm, 24.60 µm and 21.52 µm, respectively, with an average of 31.78 µm.

The width of stomata was recorded in 2^{nd} date 627 nm, 870.3 nm, 577 nm, 816 nm and 1.518 µm, with an average of 881.7 nm for heat tolerance genotype DBW-150, GW-463, WH-1179, J-2015-03, and HD-3118, respectively. In heat susceptible genotype WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725 the width was recorded 2.261 µm, 1.891 µm, 888.6 nm, 1.130 µm and 919.3 nm, respectively, with an average of 1418 nm.

Discussion

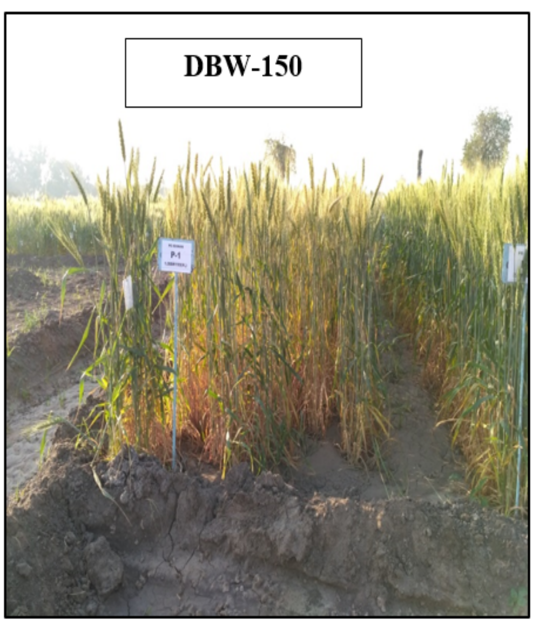
Scanning electron microscopy of wheat leaves showed difference in stomata structure in all genotype. However, in 2^{nd} sowing date the mean length and mean width of stomata were decreases in tolerant genotypes DBW-150, GW-463, GW-1179, J-15-03, and HD-3118 compared to 1^{st} date. In heat susceptible genotypes WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725 the mean length and mean width of stomata were increases in 2^{nd} date as compared to 1^{st} date. The significant difference in stomata length and width among the heat tolerance and susceptible genotypes was noticed (Ramani *et al.*, 2016) [9]. Interestingly enough the same was also reported during drought for stomata length and stomata width (Woodward, 1987) [12].

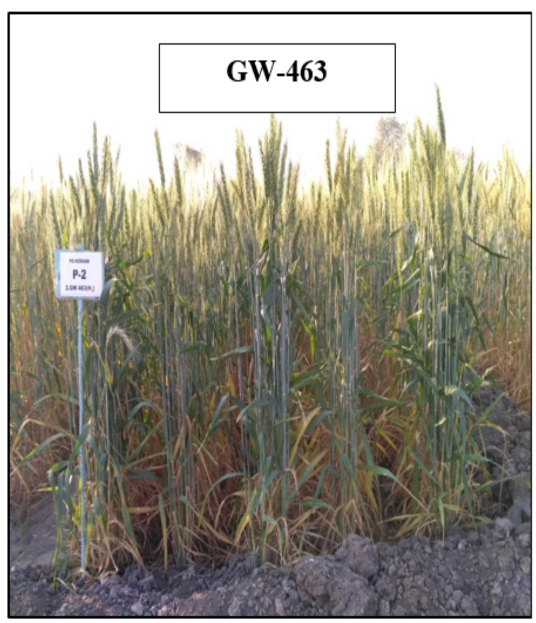
The responses of stomatal density (SD, the number of stomata per unit area) and stomatal index (SI, the proportion of stomata in relation to total number of epidermal plus stomatal cells) to global warming are important in determining the potential efficiency of leaf-level gas exchange, and thus ecosystem carbon cycles under future global warming (Apple *et al.*, 2000) [2].

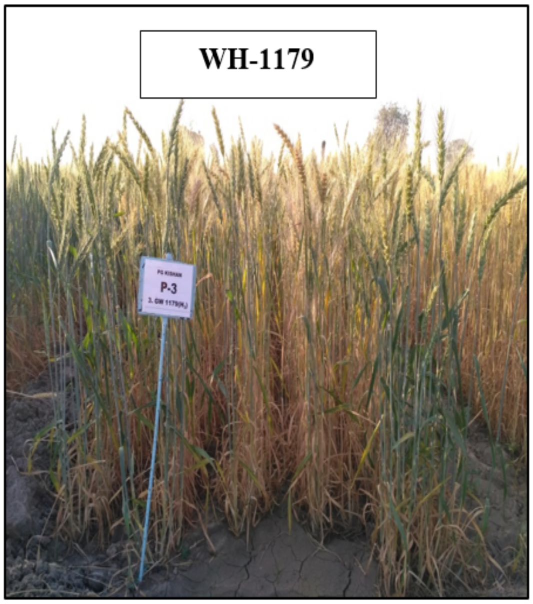
The effects of temperature on stomata aperture vary among different kinds of plants (Meyer and Anderson, 1952) [7]. In contrast, Wuenscher and Kozlowski (Wuenscher and Kozlowaski, 1971) [13] found that in five Wisconsin tree species stomata opening was effected significantly as the temperature increased from 20 to 40°C, the change was the maximum for trees that normally grow on dry sites.

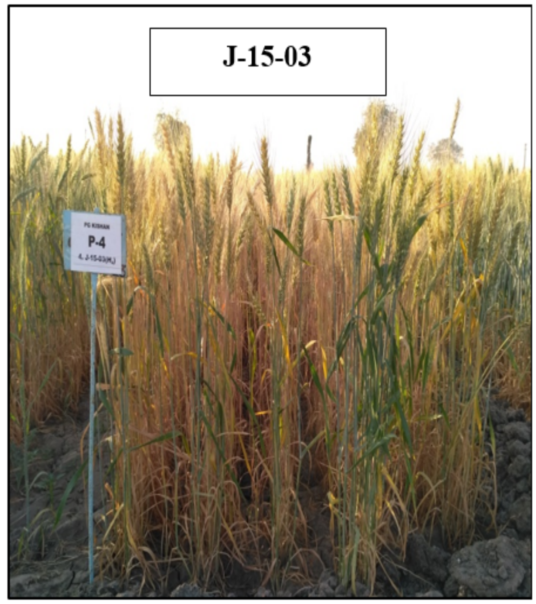
Conclusion

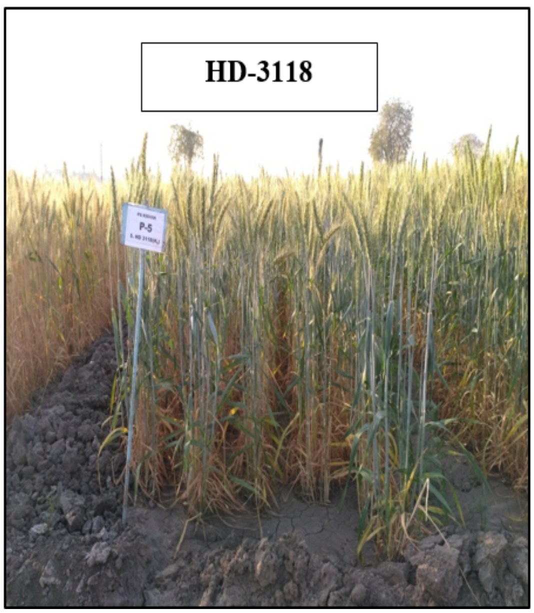
It was concluded that the late sowing effect of high temperature on stomata was more in heat susceptible genotypes (WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725) as compared to heat tolerance genotype (DBW-150, GW-463, WH-1179, J-2015-03, HD-3118). So it was suggested that DBW-150, GW-463, WH-1179, J-2015-03, and HD-3118 a heat tolerance genotype was more suitable in higher temperature region.

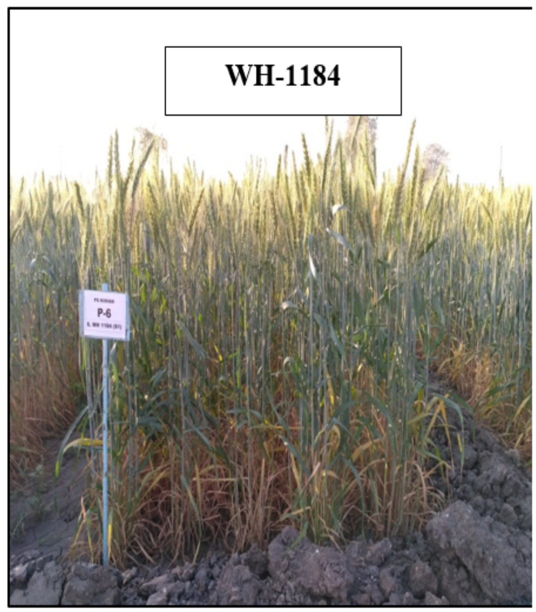


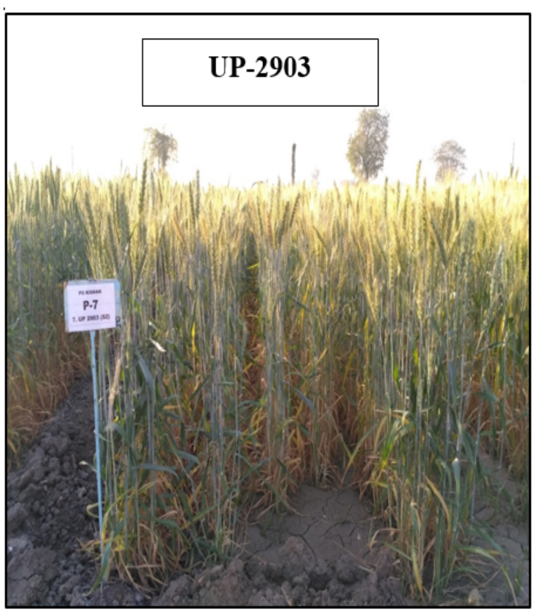


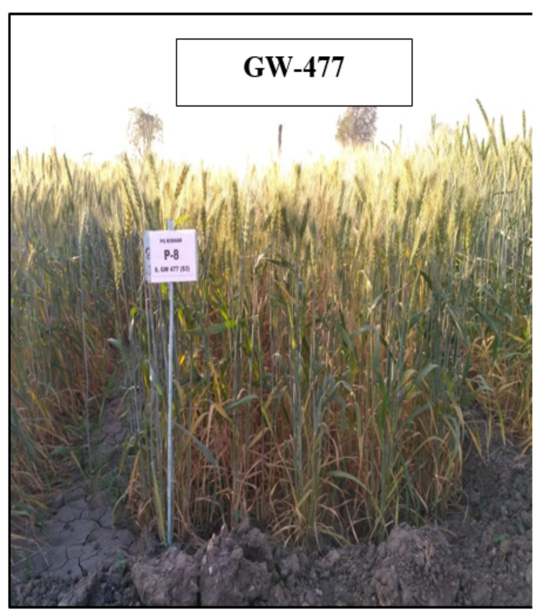

WH-1179
P-3


J-15-03
P-4


HD-3118
P-5


WH-1184
P-6


UP-2903
P-7


GW-477
P-8

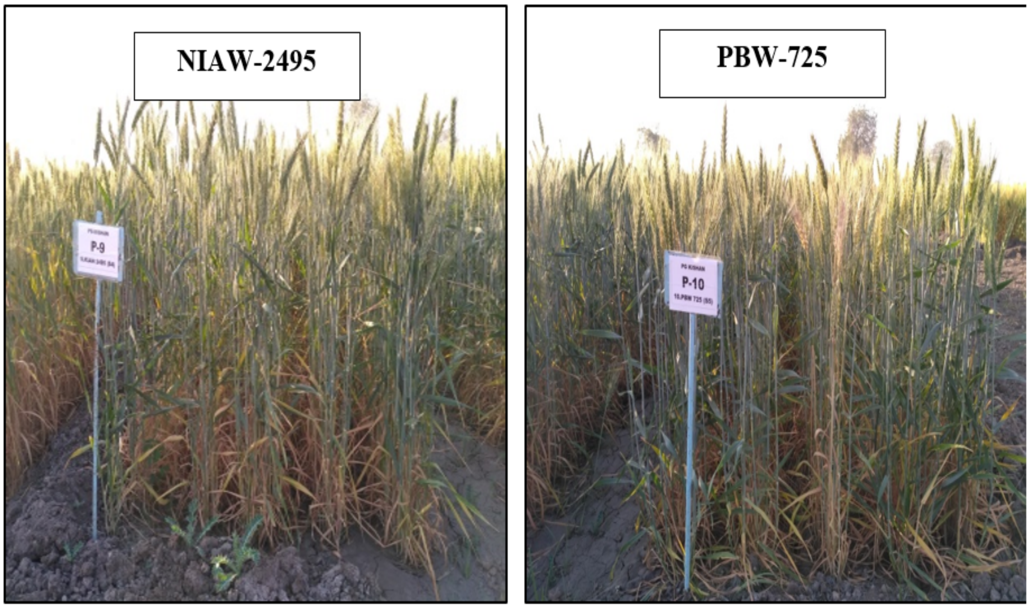
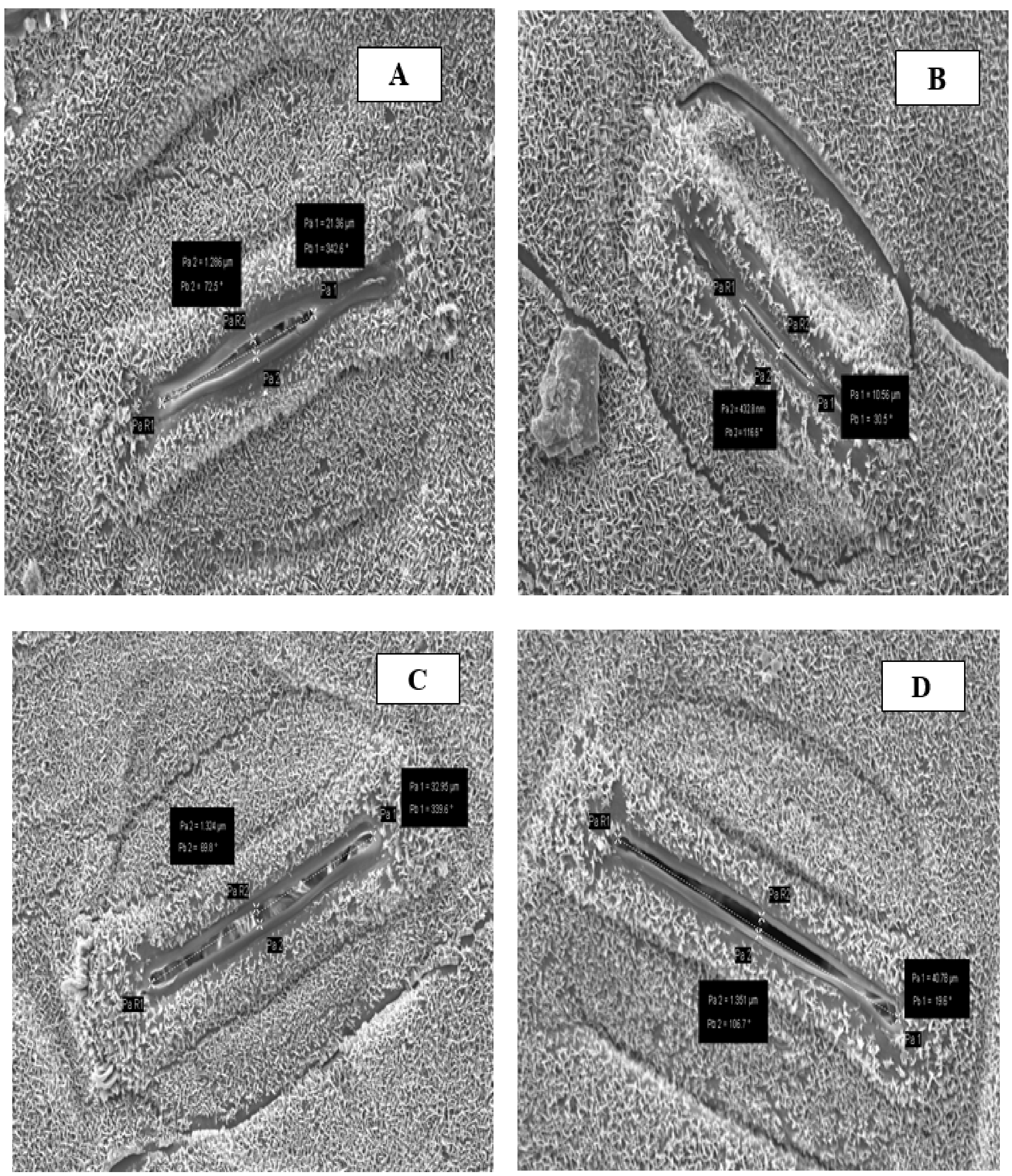


Fig 1: Heat tolerance (DBW-150, GW-463, WH-1179, J-2015-03, HD-3118) and heat susceptible (WH-1184, UP-2903, GW-477, NIAW-2495, PBW-725) genotype grown in field condition.



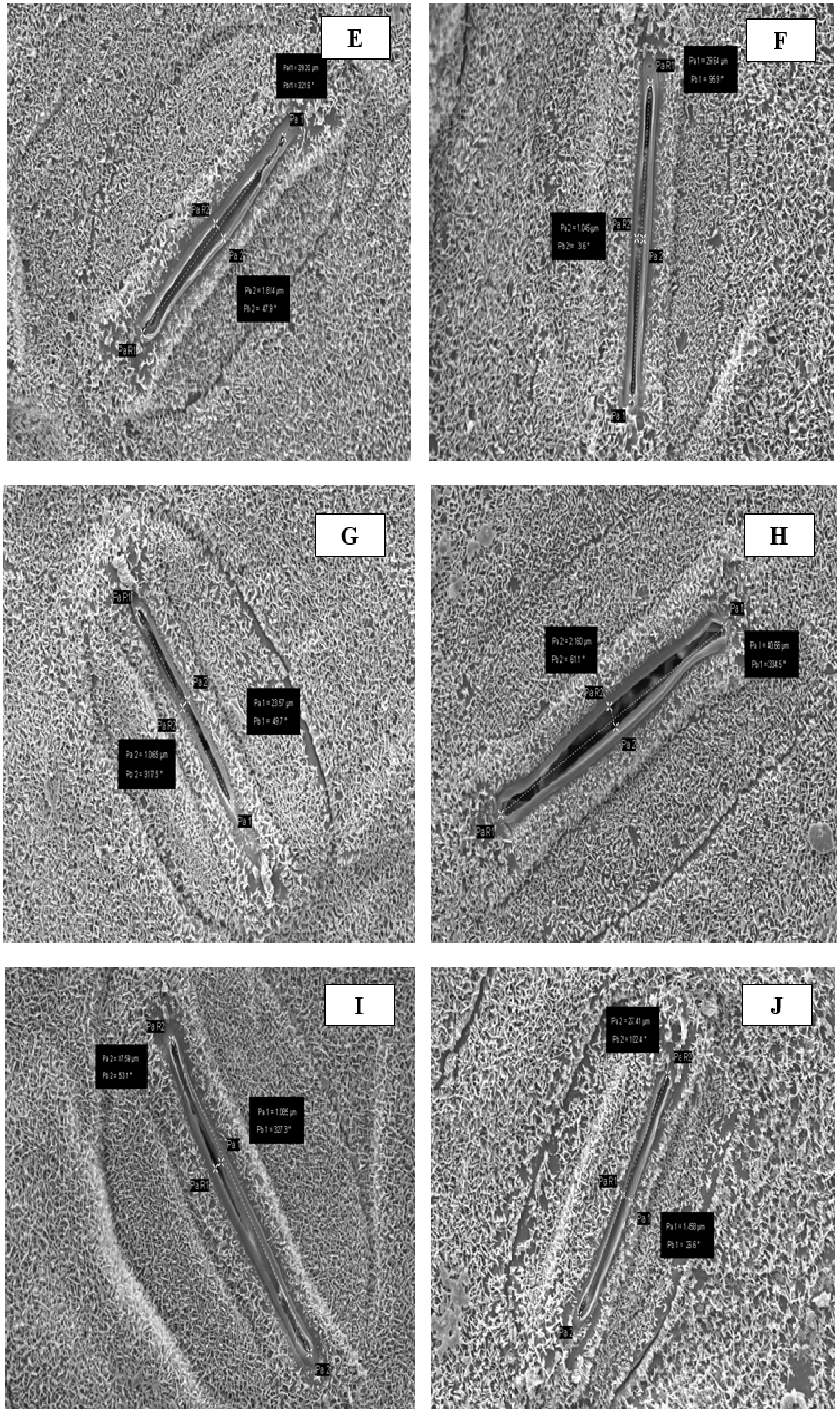
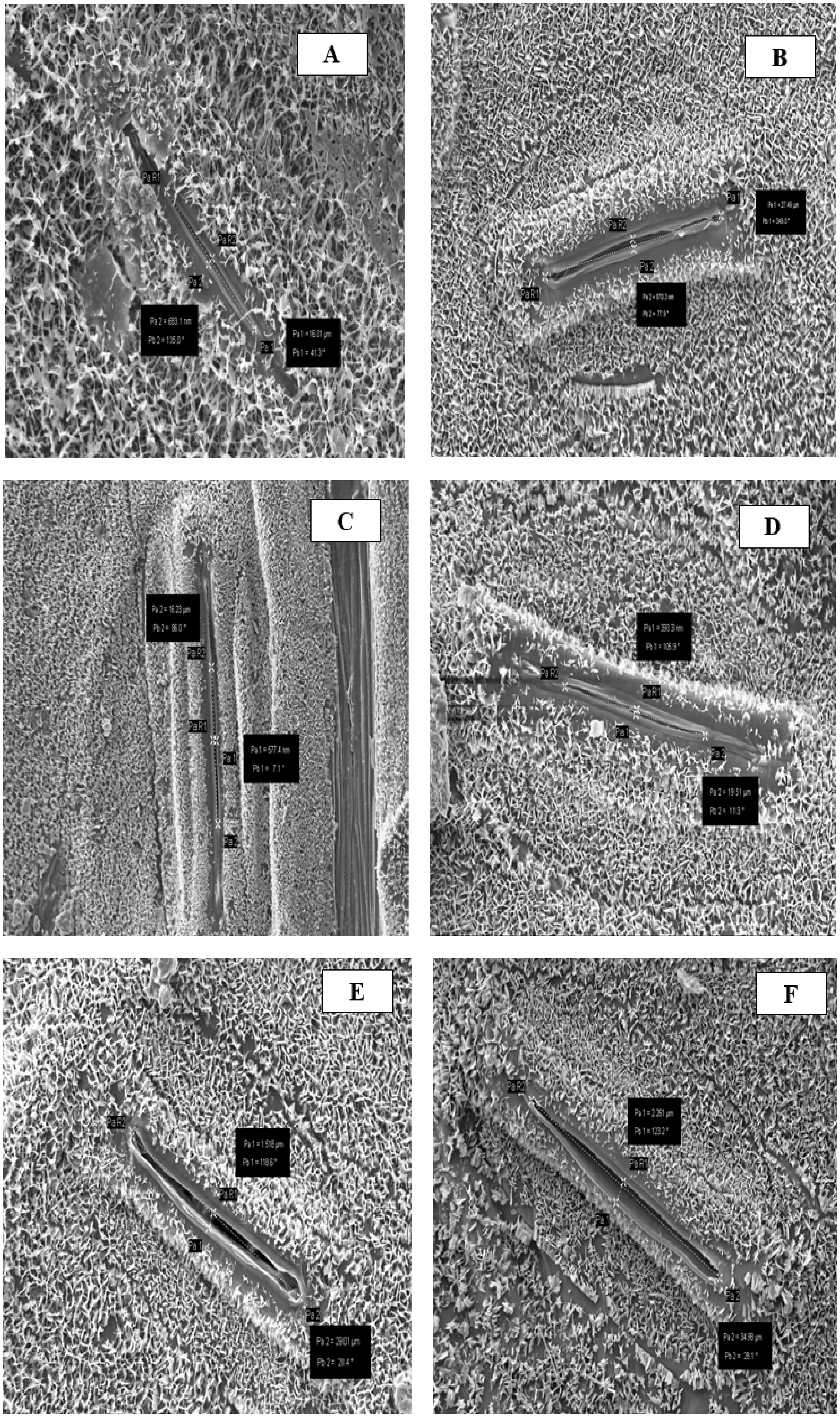


Fig 2: Effect of the temperature on stomata size of DBW-150(A), GW-463(B), WH-1179,(C) J-2015-03(D), HD-3118(E) heat tolerance genotype and WH-1184(F), UP-2903(G), GW-477(H), NIAW-2495(I), PBW-725(J) heat susceptible genotype under scanning electron microscope in 1st date showing(28th November).


A
B
C
D
E
F

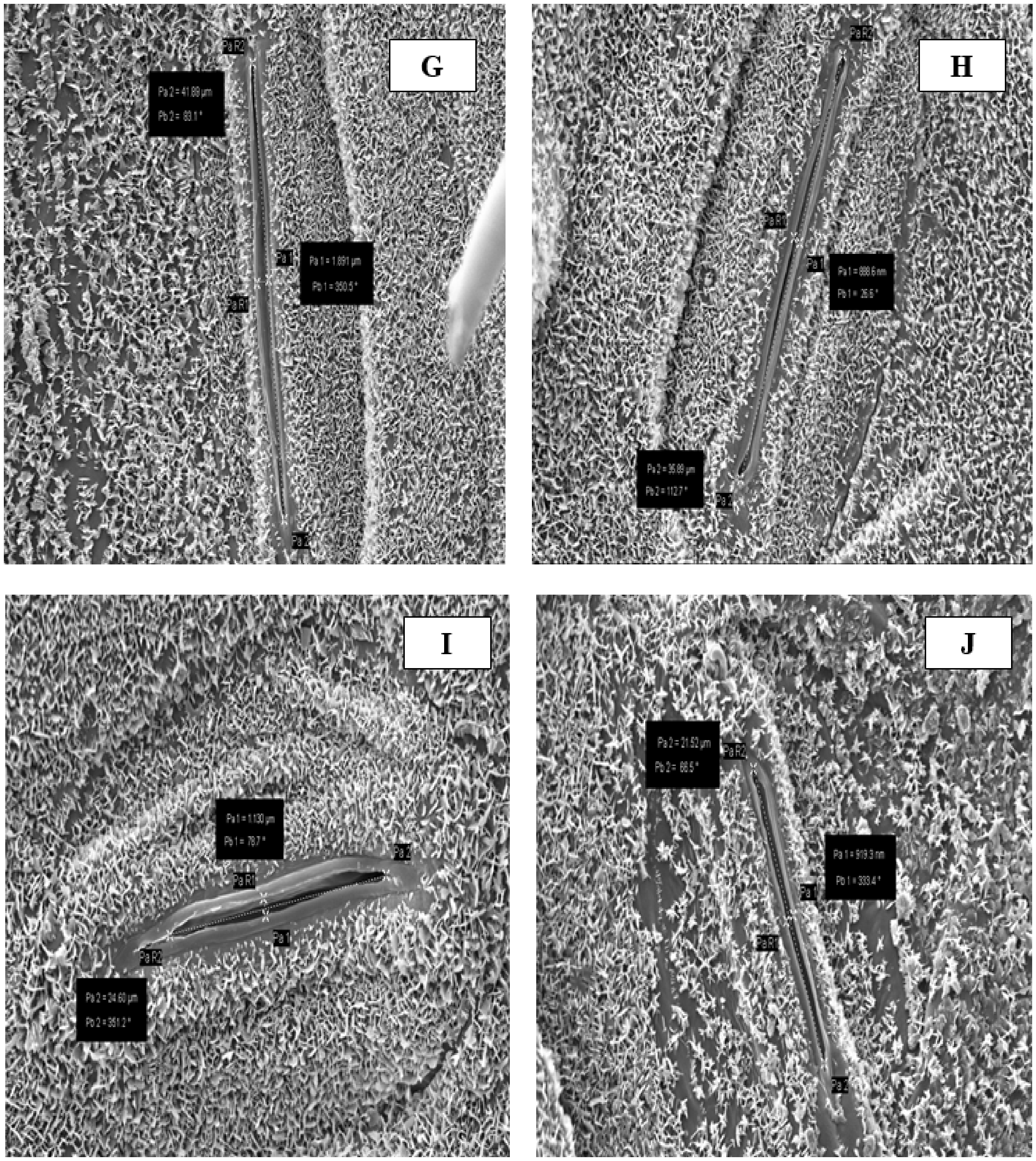


Fig 3: Effect of the temperature on stomata size of DBW-150(A), GW-463(B), WH-1179,(C) J-2015-03(D), HD-3118(E) heat tolerance genotype and WH-1184(F), UP-2903(G), GW-477(H), NIAW-2495(I), PBW-725(J) heat susceptible genotype under scanning electron microscope in 2nd date showing (31st December).

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