

GENOTYPIC VARIATION IN STOMATAL TRAITS IN LEAVES OF MAIZE

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ABSTRACT: Stomatal traits viz., stomatal density and size variation (length and width) were studied in twenty seven maize inbred lines along with two varieties (varunand Harsha) and one hybrid (DHM-117). Stomatal traits assessed amongst maize lines revealed significant variations for stomatal density, stomatal length and width on adaxial and abaxial surface and adaxial/abaxial ratio of stomata density. This information may be utilized for physiological studies, efficient breeding and crop improvement in maize.

Key words: Maize, Stomatal density, stomatal length and width, adaxial/abaxial ratio of stomata

Abbreviations: Stomata density (SD), Stomatal length (SL), Stomatal width (SW)

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INTRODUCTION

Stomata, the small pores on the surfaces of leaves and stalks that are bounded by a pair of guard cells, are the main portals of gas exchange between a plant's above-ground organs and the atmosphere. Stomata control the movement of gases in and out of a leaf, make carbon dioxide available for photosynthesis, and control the loss of water from the leaf during transpiration. Gas exchange is regulated by controlling the aperture of the stomatal pore and the number of the stomata (density) that form on the epidermis. The density of the stomata determines the stomatal conductance to CO₂ and H₂O because gaseous diffusion is regulated through turgor-mediated variation in the aperture of stomatal pores.

Stomatal size, density and distribution differ significantly among different genotypes (Jones, 1979; Upreti *et al.*, 2002; Zheng *et al.*, 2006) as well as between leaf surfaces (Ricciardi, 1984) notwithstanding the importance of stomata as a useful marker to identify genotypes (Ghosh *et al.*, 2004). Stomatal spacing (Hazra *et al.*, 1991) and aperture size (Tsuno and Sugumoto *et al.*, 1981) are reported to influence photosynthetic rate positively (Medlyn *et al.*, 2001; Xu and Baldocchi, 2003). Further, comparison of stomatal frequency between leaf surfaces is being related to drought resistance (Hu, 1989; Vaz *et al.*, 2010) and this may be one of the useful criteria in screening for drought tolerant genotypes (Shawesh *et al.*, 1985; Munns *et al.*, 2010).

Maize (*Zea mays* L.) is an economically important food crop. Few studies have reported variation in stomatal traits of maize leaves due to plant growth and growth media (Orce *et al.*, 2013), warming effects (Zheng *et al.*, 2013) and soil water deficit (Zhao *et al.*, 2015). As limited information is available on stomatal traits in maize genotypes, the objective of the present study was to determine stomatal density and size (length and width) on abaxial and adaxial surfaces of leaves which may be utilized for physiological studies, efficient breeding and crop improvement.

MATERIALS AND METHODS

The research work was conducted at ICAR-Central Research Institute for Dry land Agriculture (ICAR-CRIDA), Santoshnagar, Hyderabad, India, during 2013 summer season. The experimental material of 27 genotypes of maize used in the present study, namely HKI-161T, HKI-163, HKI-164-7-4, HKI-165, HKI-1035-10, HKI-1011, HKI-3-4-6ER, HKI 1025, HKI 209, HKI 1332, HKI 766(0), HKI 577, HKI 1040-4, HKI 488, HKI 46, HKI 325-17AN, HKI 1105, HKI 47, HKI 295, HKI 659-3, HKI 288-2, HKI-L287, LM5, LM6, LM13, LM14, LM16 were inbred lines from ICAR-Directorate of Maize Research, New Delhi. Twenty seven maize genotypes from ICAR-DMR along with one hybrid DHM-117 and two varieties *viz.*, Harsha and Varun were grown in pots under well-watered condition. The experiment was laid out in a randomized complete block design with three replications. Recommended dose of fertilizer was used and standard agronomic practices were adopted. Stomatal observations were carried out between 45- 48 days after sowing (DAS). The middle part of second youngest fully expanded leaves from the top of plants was selected from each genotype for counting the stomata number/mm² (density) and size on upper and lower surface of leaf. Two plants per genotype and one leaf per plant and four areas per leaf were examined. Stomatal size (length and width) was recorded for ten stomata per leaf.

After collecting, the leaves were washed and placed on glass slide, peeled off by scraping the leaf epidermis gently with the help of sharp blade. These epidermis peeled leaf samples were taken on to the glass slide, washed with water and then mounted in a drop of glycerin on to the sample to prevent drying. A cover-slip was placed and excess of water were blotted with the help of blotting paper. Digital images of both adaxial and abaxial side of leaves were obtained using a Sterio microscopic (Olympus-szx10) equipped with Prog Res^(R) microscope camera. Four square areas (a total of 4 mm²) and length and width (μm) of ten stomata on both abaxial and adaxial surface were recorded in each leaf by processing the digital images using Prog Res Capture software v. 2.7 (Jenoptik Optical Systems) in different genotypes. To find the stomatal density, the number of stomata per one mm² of leaf (No./mm²) were counted from the images. The adaxial/abaxial ratio of stomata density was calculated. Stomata length was measured between the junctions of the guard cells at each end of the stomata and width was measured perpendicular to maximum width which represents the maximum potential opening of the stomatal pore. The experimental data were statistically analyzed (ANOVA) to assess significant variation among the genotypes for attributes like stomatal density and size.

RESULTS AND DISCUSSION

Results of stomatal traits are presented in (Fig. 1-3). Analysis of variance indicated that significant differences existed among the maize genotypes for stomatal density (SD) and size (length and width) on both adaxial and abaxial surfaces of leaf.

Difference between inbred lines in terms of stomatal density on both abaxial and adaxial surfaces of leaf was also significant. Generally all cultivars had more stomata on the abaxial compared to adaxial surface (Fig. 1). Genotypes HKI-1011 and HKI-164-7-4 had maximum stomata density of 99/mm² and 89/mm² on abaxial surface and 74 and 76/mm² on adaxial surface respectively. The genotypes HKI-1035-10 and LM-13 had minimum density of 24 and 50/mm² on abaxial surface and 21 and 34.5/mm² on adaxial surface respectively. A cultivated variety *viz.* varun had a lower stomatal density of 42.5/mm² and 28.5/mm² on adaxial and abaxial surface respectively while a popularly grown hybrid had higher stomatal density of 104/mm² and 77/mm² on abaxial and adaxial surface. Similarly significant intraplant variation in stomatal frequency, size and total stomatal area per unit leaf area was reported in tetraploid and hexaploid wheat (Whang *et al.*, 1993), in terms of transpiration rate, stomatal frequency, stomatal size in wheat cultivars (kobra *et al.*, 2008) and stomatal characteristics of wheat wild species and land races (Khazaei *et al.*, 2010).

The ratio between the number of adaxial and abaxial stomata of HKI-L-287(0.95), HKI-3-4-6ER (0.93) and HKI-46 (0.917) was close to 1 and ratio of LM-6 (0.437), LM-14 (0.539) and HKI-1025 (0.58) was close to 0.5. Comparison of stomatal frequency between leaf surfaces is being related to drought resistance (Hu, 1989; Vaz *et al.*, 2010) and this may be one of the useful criteria in screening for drought tolerant genotypes (Shawesh *et al.*, 1985; Munns *et al.*, 2010).

The significant differences found between maize genotypes in terms of stomatal length and width on both the adaxial and abaxial surface of leaf implied genetic variation between lines (Fig. 2&3). The stomata length ranged between 33.79 (HKI-1105) and 56.74 (HKI 766(0)) on abaxial surface and 28.47 (HKI-1105) and 56.95 (HKI-3-4-6ER) on abaxial surface. The stomatal width ranged between 26.57 μm (LM 5) and 42.46 (HKI 1040-4) on abaxial surface and 28.70 (HKI 295) and 38.45 (HKI-1025) on adaxial surface.

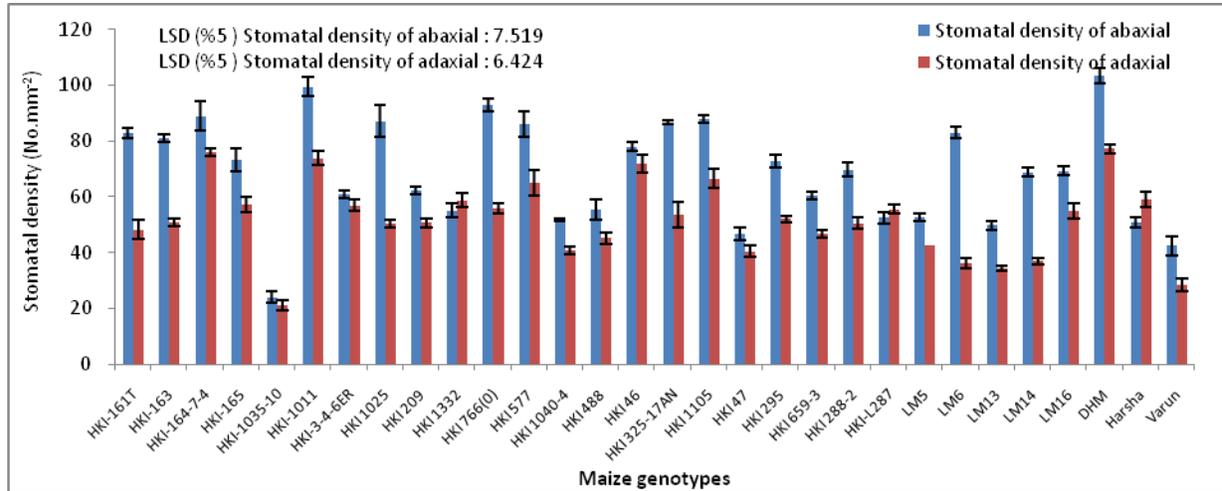


Fig 1. Mean values of Stomatal density on abaxial and adaxial surface of leaves in maize lines

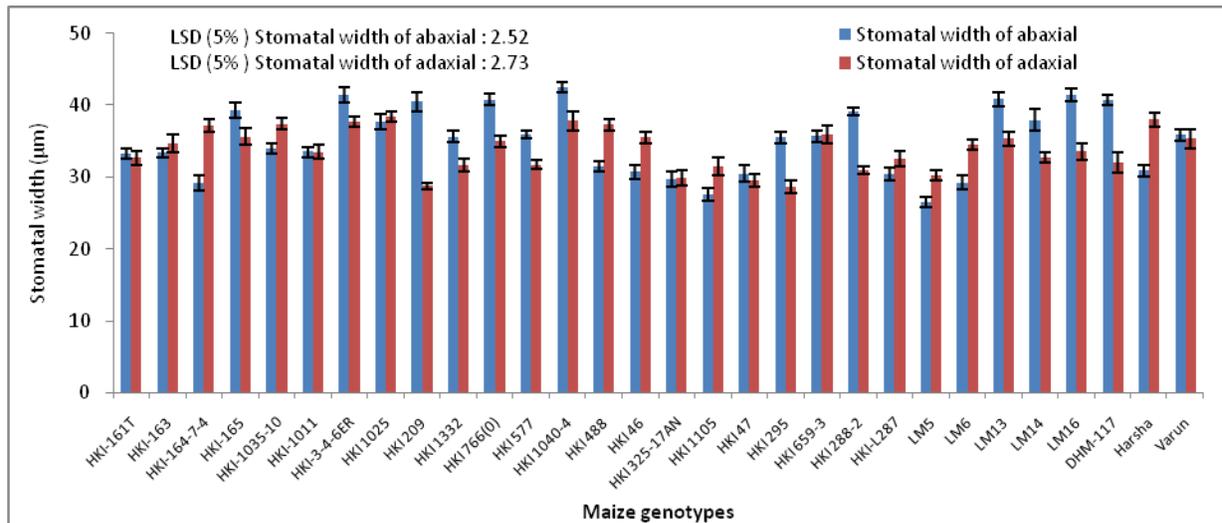


Fig. 2: Mean values of stomatal width on abaxial and adaxial surface of leaves in maize lines.

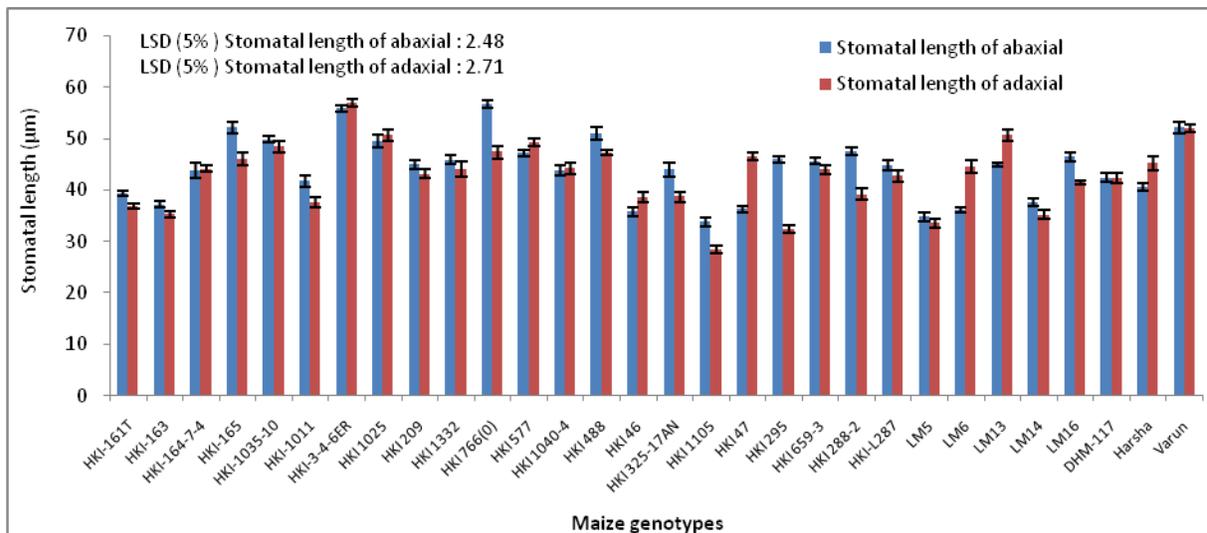


Fig. 3: Mean values of stomatal length on abaxial and adaxial sides of leaves in maize lines.

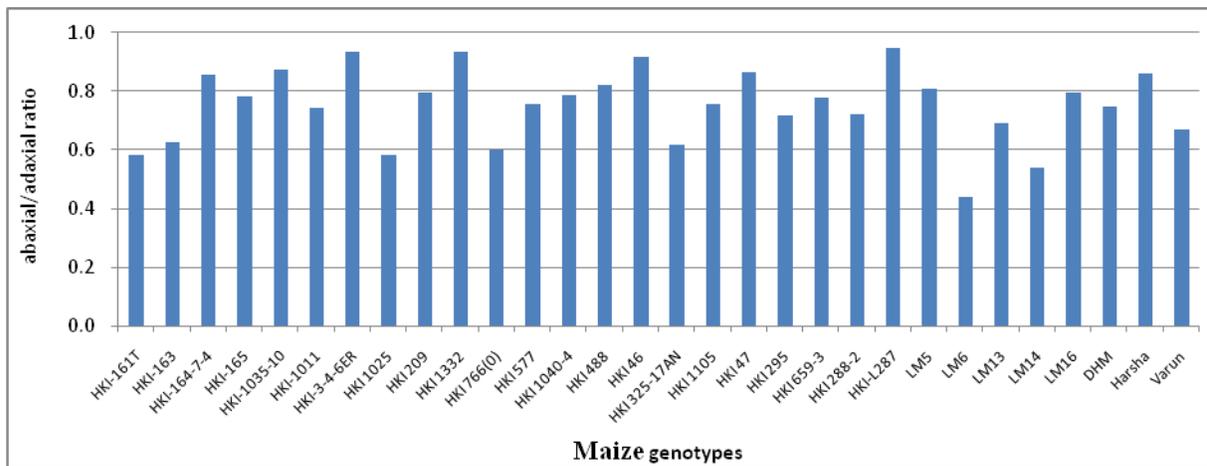


Fig. 4: Mean values of adaxial/abaxial ratio of stomatal density in maize lines.

CONCLUSION

The present study conclusively indicated that the maize genotypes differed in stomatal density, size (length and width) and adaxial/abaxial ratio of stomata density. Stomatal density and size along with yield and moisture stress studies can be a useful tool for characterizing the drought tolerant lines and selection of high yielding and drought-tolerant genotypes.

ACKNOWLEDGMENTS

This work was carried out under the National Initiative on Climate Resilient Agriculture (NICRA) project launched by Department of Agricultural Research and Education, Govt. of India, through Indian Council of Agriculture Research, New Delhi, India. I thank Directorate of Maize Research-ICAR, for providing the maize seeds.

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ISSN : 0976-4550

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