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Response of maize (*Zea mays* L.) on yield, physiology and stomatal behaviour under two different elevated CO₂ concentrations. Do these anatomical changes affect the physiology of the C4 crop plant under high CO₂ conditions?

IRA KHAN^{1*}, MADDI VANAJA², POLDASARI SATHISH², MOHAMMAD FAIZAN¹, SIPAN SOYSAL³, VISHNU D. RAJPUT⁴, IVICA DJALOVIC⁵, GORAN TRIVAN⁶, PRAVEJ ALAM^{7*}

¹Botany Section, School of Sciences, Maulana Azad National Urdu University, Hyderabad, India

²Central Research Institute for Dryland Agriculture, Santoshnagar, Hyderabad, India

³Department of Field Crops, Faculty of Agriculture, Siirt University, Siirt, Türkiye

⁴Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don, Russia

⁵Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia

⁶Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia

⁷Department of Biology, College of Science and Humanities, Prince Sattam Bin Abdulaziz University, Alkharj, Saudi Arabia

*Corresponding authors: irakhan@manuu.edu.in; alamprez@gmail.com

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Abstract: Rising CO₂ concentration in the atmosphere is a matter of global concern and poses apprehension about how plants will adapt to the changing environment. Various studies have proved that under high CO₂ levels, plant physiology alters and affects plant functioning. However, under elevated CO₂, the stomatal characters and their relation with physiological responses are still not yet clear. To find out these changes in the stomatal parameters at ambient and two elevated CO₂ (550 ppm and 700 ppm) levels, four genotypes of maize (*Zea mays* L.) viz. DHM-117, Harsha, Varun and M-24 were grown in open-top chambers. In the study, it was observed that the stomatal density increased, stomatal size altered, stomatal conductance (g_s) and transpiration rate (T_r) decreased under elevated CO₂ (eCO₂) while photosynthetic rate (P_n), water use efficiency (WUE), yield and biomass, of which especially the reproductive biomass increased. Under eCO₂, stomatal and physiological changes were genotypic and CO₂ concentration specific. Increased stomatal density at eCO₂ was mainly due to increased abaxial stomatal density. The improved P_n and reduced T_r at 550 ppm improved the WUE in the plants, while this response was not observed at 700 ppm. These results elucidate that this C4 crop responded positively to up to 550 ppm of CO₂ concentrations, and beyond this, the impact was minimal.

Keywords: grain number; greenhouse gases; stomatal conductance; photosynthesis; cereals; climate change

The change in climate with increasing concentrations of atmospheric CO₂ and other greenhouse gases is a global concern as it can pose a great challenge for sustainable food production in future. A primary factor contributing to climate change is higher atmospheric

carbon dioxide concentration or elevated CO₂. It has risen from the pre-industrial level of 280 µmol/mol in 1750 to approximately 400 µmol/mol at this time, and by the end of the 21st century, it is predicted to reach approximately 900 µmol/mol (Xu et al. 2016).

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Stomata are the pores on the leaf surface controlling gaseous exchange, mainly CO₂ and water vapour, between the atmosphere and plants (Hetherington and Woodward 2003). Plant leaves usually optimise their gas exchange by altering stomatal pore openness, stomatal aperture size, stomatal frequency (stomatal density and stomatal index), and stomatal distribution pattern, which are regulated by both environmental factors (Lake et al. 2002, Hetherington and Woodward 2003, Lake and Woodward 2008, Franks and Beerling 2009) and genetic signals (Bergmann 2004). It has been demonstrated by various experiments that growing plants under an elevated CO₂ regime in controlled environmental growth chambers results in leaves with a decreased stomatal density (Thomas and Harvey 1983, Woodward 1987). Stomatal conductance (g_s) is generally reduced in response to elevated CO₂ (Ainsworth and Rogers 2007) due to either a decrease in stomatal aperture with the reduced photosynthetic demand for CO₂ and/or a change in stomatal density (Xu et al. 2016, Yi et al. 2023). According to Drake et al. (1997), the photosynthetic rate of C3 species increases by around 58% as a result of doubling [CO₂], suggesting that their response to increased [CO₂] may be more favourable than that of C4 species. For C4 species, however, current ambient [CO₂] has almost completely saturated photosynthesis (von Caemmerer et al. 1997). Conversely, it has been anticipated that C4 species will fare better than C3 species in greater temperatures and droughts (Long 1999). The reason for this is because in C4 species, when stomatal conductance is decreased and water availability is limited, the plants can maintain the rate of CO₂ assimilation due to the CO₂ concentrating mechanism (CCM) (Knapp and Medina 1999, Xu et al. 2022). According to Ziska's (2001) analysis, sorghum competitiveness against *Xanthium strumarium* L. declined as CO₂ concentration increased, both under normal and elevated CO₂ conditions.

Globally, maize (*Zea mays* L.) is known as the queen of cereals because it has the highest genetic yield potential among the cereals. It is the world's third most important food crop in terms of production, and its demand is predicted to increase drastically soon (Young and Long 2000). Considering previous research, we designed our experiment to study changes in stomatal, physiological, and yield-related parameters under elevated CO₂ conditions in *Z. mays* and work out the correlation between the different anatomical and physiological parameters.

MATERIAL AND METHODS

Seeds of four maize genotypes *viz.* DHM-117, Harsha, Varun and M-24, obtained from the DMR regional station at Hyderabad, India, were grown in open-top chambers (OTCs) of 3 m × 3 m × 3 m dimensions at the Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad, India. The city is geographically situated at 17°19'52.549" to 17°21'42.986"N latitude and 78°35'03.295" to 78°36'46.990"E longitudes. The area comes under a semi-arid (dry) climate with well-expressed summer (March to May), rainy (June to September) and mild winter (November to February). This experiment was conducted in 2018–2019, and during the experiment, the air temperature was 25 °C, photosynthetic photon flux density was 800 μmol/mol²/s, relative humidity was 85%, and ambient CO₂ concentration was 405 ppm. The soil was Alfisol in nature and containing 0.37% organic carbon, 14.5 cmol₊/kg CEC, 210 mg total P/kg, 65 mg available N/kg, 5.6 mg available P/kg and 80 mg available K/kg.

The open-top chambers were correctly maintained in an open field. The OTCs with 3 m × 3 m × 3 m dimensions lined with transparent PVC (polyvinyl chloride) sheets having 90% light transmittance were used. The elevated CO₂ of 550 ppm and 700 ppm were maintained in four OTCs, and the other two OTCs, without any additional CO₂ supply, served as ambient control. The CO₂ concentrations within the OTCs were maintained and monitored continuously throughout the experimental period, as Vanaja et al. (2006) illustrated. Experiments were conducted in 6 open-top chambers. Among the six OTCs, two chambers were maintained without any external CO₂ supply, which served as ambient control (aCO₂) conditions. The maize plants grown in the other four OTCs were continuously supplied with CO₂ to maintain elevated CO₂ conditions (eCO₂) of 550 ppm [550 ppm] in two and 700 ppm [700 ppm] in the other two chambers. The seeds of maize were directly sown in the soil inside each OTC. The spacing between the plants was maintained at 0.35 m, and between the rows, it was 0.75 m. In each chamber, 24 plants were planted, and 6 plants were planted for each genotype. The recommended dose of fertiliser was applied from time to time, and the crop was irrigated at regular intervals. The recommended dose of fertilisers was 60 kg N/ha and 60 kg P/ha as diammonium phosphate, 30 kg K/ha as muriate of potash was applied as basal dose; a second dose of

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30 kg N/ha at knee-high stage and a third dose of 30 kg N/ha as urea and 30 kg potassium/ha as muriate of potash was side dressed at tasseling stage. The crop was irrigated at regular intervals and maintained pest and disease-free with plant protection measures. The measurement was taken on a fully expanded third leaf from the uppermost active leaf in three representative plants for each maize genotype. The measurements were recorded between 10:00 and 12:00 h using a portable photosynthesis system (LI-6400, LI-COR, Nebraska, USA) with irradiance set at 1 200 $\mu\text{mol}/\text{m}^2/\text{s}$ under three conditions *viz.*, aT, eT, and eT + eCO₂.

For recording the stomatal parameters, the third leaf from the top of the selected plant from all treatments was sampled during anthesis, and precautions were taken to draw the sample from the same position as the leaf. Four plants of each genotype were selected from each treatment, and with the help of the acetate peel method (Beerling and Chaloner 1992), the samples were prepared to analyse the stomatal parameters under a high-resolution microscope. The micrographs obtained were used to record stomatal density (SD). Four replications with one mm² leaf area were selected for SD, and ten observations for stomatal width (SW) and stomatal length (SL) were recorded on both leaf surfaces. Both adaxial (upper) and abaxial (lower) surfaces of leaves were studied for stomatal density, which was calculated as the total number of stomata per mm² area of the leaf. Total stomatal density (TSD) was calculated as the total number of adaxial and abaxial stomata per mm² of leaf. The stomatal size, i.e. stomatal width and stomatal length, was measured using Olympus-SZX-10 microscope (India) and is expressed in μm .

The physiological parameters like photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (T_r) were recorded with a portable photosynthesis system (LICOR-6400). The water use efficiency (WUE) was calculated as the ratio of P_n and T_r . Plant height, dry biomass, and yield indices were measured at harvest, including cob weight (g/pl), number of grains/cob, grain weight (g/pl), 100-grain weight, and harvest index (HI) of all four genotypes. These data were recorded in three replications for each treatment. Vegetative and reproductive biomass made up the total biomass. The total dry biomass of the root, leaf, and stem was used to compute the vegetative biomass, and the weight of the cob was used to calculate the reproductive biomass. The change in the response of these parameters due to eCO₂ was

quantified as a percentage increase from ambient. A two-way analysis of variance (ANOVA) was used to analyse the data statistically. The study employed correlation coefficient analysis to examine the link between stomatal and physiological variables.

RESULTS

The performance of stomatal parameters of four maize genotypes – Harsha, Varun, DHM-117 and M-24 was quantified at ambient CO₂ (aCO₂) and elevated CO₂ (eCO₂) conditions of 550 ppm and 700 ppm. The parameters recorded are adaxial surface stomatal density (AdSD), abaxial surface stomatal density (AbSD), adaxial surface stomata width (AdSW), adaxial surface stomata length (AdSL), abaxial surface stomata width (AbSW), abaxial surface stomata length (AbSL), total stomatal density (TSD) and adaxial/abaxial ratio (Ad/Ab).

The ANOVA of stomatal density and size revealed that genotypes (G), CO₂ levels (C) and G $\times$ C varied highly significantly ($P < 0.01$) for AdSD, AbSD, AdSW, AbSW and AbSL. At the same time, AdSL is non-significant for CO₂ levels (Table 1).

Stomatal parameters

Adaxial surface stomatal density. The number of stomata on the upper leaf surface was altered when the plants were grown in elevated CO₂. In genotype M-24, the stomatal density decreased by 8.52% and 20.17% under 550 ppm and 700 ppm, respectively, over aCO₂. In Harsha, the decrease was 3.23% at 550 ppm, and an increase of 19% was observed under 700 ppm. The decrease observed in genotype Varun was 2.84% and 20.92% under 550 ppm and 700 ppm, respectively, over aCO₂. In DHM-117, the density increased by 24.49% under 550 ppm while decreasing by 4.42% under 700 ppm over aCO₂.

Abaxial surface stomatal density. Variation was observed in the lower surface of leaf stomatal number as in M-24; it was 99/mm², 103.8/mm² and 71.3/mm² under aCO₂, 550 ppm and 700 ppm, respectively. In Harsha, it was 82.75/mm², 102/mm² and 93.75/mm² under aCO₂, 550 ppm and 700 ppm, respectively. The AbSD of Varun was 91.5/mm², 105.0/mm² and 87.75/mm² under aCO₂, 550 ppm and 700 ppm, respectively. The AbSD of DHM-117 was 89.75/mm², 103.2/mm² and 110/mm² under aCO₂, 550 ppm and 700 ppm, respectively (Table 2).

Total surface stomatal density (TSD). The total stomatal density was changed in elevated CO₂

Table 1. Analysis of variance of the stomatal and physiological parameters of four maize genotypes under three CO₂ concentrations

	Mean sum of squares									
	AdSD	AbSD	AdSW	AdSL	AbSW	AbSL	P _n	WUE	g _s	T _r
df	3	3	9	9	9	9	5	5	5	5
Replication (R)	36.611	32.472	9.945	9.712	7.83	6.245	34.121	0.284	0.001	0.972
Genotype (G)	534.00**	263.472**	919.788**	141.754**	482.752**	343.617**	101.862**	1.847**	0.031**	6.653**
CO ₂ levels (C)	228.646**	967.521**	131.84**	0.007 ^{ns}	88.799**	227.787**	177.991**	53.545**	0.192**	45.723**
G × C	383.229**	475.826**	189.04**	49.023**	104.235**	148.156**	24.221*	0.748*	0.010**	1.610*
Error	16.338	34.487	6.964	7.319	8.356	8.218	9.852	0.241	0.001	0.426
Total	106.546	145.020	41.48	12.869	26.462	27.27	21.399	1.857	0.008	2.103

* $P < 0.05$; ** $P < 0.01$; ns – non significant; AdSD – stomatal density on adaxial surface; AbSD – stomatal density on abaxial surface; AdSW – adaxial stomata width; AdSL – adaxial stomata length; AbSW – abaxial stomata width; AbSL – abaxial stomata length; P_n – photosynthetic rate; g_s – stomatal conductance; T_r – transpiration rate; WUE – water use efficiency

conditions. The decrease in TSD was observed in M-24, which was 1.47% and 24.29% under 550 ppm and 700 ppm, respectively, over aCO₂. The TSD of Harsha increased by 11.15% and 15.9% due to 550 ppm and 700 ppm over aCO₂, respectively. The same parameter in Varun showed an increase of 7.1% due to 550 ppm while a decrease of 11.42% due to 700 ppm over aCO₂. The TSD of DHM-117 increased by 20.98% and 10.41% due to 550 ppm and 700 ppm over aCO₂, respectively.

Ad/Ab ratio. The Ad/Ab ratio of M-24 was 0.89, 0.78 and 0.98 under aCO₂, 550 ppm and 700 ppm, respectively. Harsha's Ad/Ab ratio was 0.84, 0.66 and

0.89 under aCO₂, 550 ppm and 700 ppm, respectively. This ratio in Varun was 0.77, 0.65 and 0.64 under aCO₂, 550 ppm and 700 ppm, respectively, while in DHM-117, it was 0.82, 0.89 and 0.64 under aCO₂, 550 ppm and 700 ppm, respectively.

Size of stomata

Adaxial surface stomatal width. The adaxial stomatal width also changed when the plants were grown under higher CO₂ conditions. The AdSW of M-24 was 55, 56.38 and 54.67 μm under aCO₂, 550 ppm and 700 ppm, respectively. The AdSW of genotype

Table 2. Mean *per se* values of stomatal parameters of the four maize genotypes under three CO₂ concentrations

Stomatal parameter	M-24			Harsha			Varun			DHM-117		
	CO ₂ levels											
	ambient	550	700	ambient	550	700	ambient	550	700	ambient	550	700
	(ppm)			(ppm)			(ppm)			(ppm)		
AdSD	88 ± 1.05	80.5 ± 3.45	70.25 ± 1.19	69.75 ± 2.6	67.5 ± 3.28	83.0 ± 3.06	70.5 ± 1	68.5 ± 3.96	55.75 ± 1.44	73.5 ± 2.43	91.5 ± 2.13	70.3 ± 1.28
AbSD	99 ± 2.45	104 ± 1.44	71.3 ± 0.98	82.7 ± 6.45	102 ± 6.3	93.7 ± 2.68	91.5 ± 1	105 ± 0.94	87.75 ± 0.29	89.7 ± 4.28	103.2 ± 4.77	110 ± 2.75
AdSW	55 ± 0.46	56.4 ± 1.09	54.7 ± 0.515	51.1 ± 0.76	46.9 ± 1.07	44.2 ± 1.11	39.1 ± 0.50	40 ± 0.68	47.3 ± 0.36	47.1 ± 0.93	46.48 ± 1.42	57.3 ± 1.12
AdSL	37.8 ± 0.44	35.9 ± 1.39	37.7 ± 0.52	35.2 ± 1.03	35.2 ± 0.67	30.6 ± 1.17	30.2 ± 0.93	30.9 ± 0.78	34.7 ± 0.57	34.1 ± 0.65	35.4 ± 1.2	34.3 ± 1.02
AbSW	52 ± 0.66	44.2 ± 1.26	51.6 ± 0.8	46.4 ± 1.19	44 ± 0.84	44.4 ± 0.87	41.5 ± 0.72	36.4 ± 0.81	40.6 ± 0.67	45.5 ± 0.85	48.9 ± 1.38	42.1 ± 1.1
AbSL	41.8 ± 0.89	33.53 ± 1	42.4 ± 0.9	39.2 ± 1.28	32.9 ± 1.13	32.9 ± 0.53	33.3 ± 0.72	28.2 ± 0.61	32.1 ± 0.97	38.7 ± 0.94	40.2 ± 1.05	31.4 ± 1.06

AdSD – adaxial stomatal density; AbSD – abaxial stomatal density; AdSW – adaxial stomata width (μm); AdSL – adaxial stomata length (μm); AbSW – abaxial stomata width (μm); AdSL – adaxial stomata length (μm)

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Harsha was 51.06, 46.9 and 44.15 μm under aCO_2 , 550 ppm and 700 ppm, respectively. Varun genotype was 39.06, 39.97 and 47.26 μm under aCO_2 , 550 ppm and 700 ppm, respectively. While in DHM-117 was 47.13, 46.48 and 57.29 μm under aCO_2 , 550 ppm and 700 ppm, respectively.

Abaxial surface stomatal width. A decrease was observed under elevated CO_2 conditions M-24 by 14.9% and 0.8% under 550 ppm and 700 ppm over aCO_2 , respectively. The AbSW of Harsha decreased by 5.15% and 4.32%, which was less than 550 ppm and 700 ppm over aCO_2 , respectively. The AbSW of Varun decreased by 12.44% and 2.19% at 550 ppm and 700 ppm over aCO_2 , respectively. AbSW in DHM-117 registered a 7.6% increase due to 550 ppm over aCO_2 , while a decrease of 7.5% was observed under 700 ppm over aCO_2 .

Adaxial surface stomatal length. The AdSL of M-24 was found to be 37.83, 35.92 and 37.67 μm under aCO_2 , 550 ppm and 700 ppm, respectively. The AdSL of Harsha was 35.23, 35.17 and 30.58 μm under aCO_2 , 550 ppm and 700 ppm, respectively. The AdSL of Varun was 30.18, 30.89 and 34.72 μm under aCO_2 , 550 ppm and 700 ppm, respectively. The AdSL of DHM-117 was 34.08, 35.37 μm and 34.28 μm under aCO_2 , 550 ppm and 700 ppm, respectively (Table 2).

Abaxial surface stomatal length. The AbSL of M-24 decreased by 19% due to 550 ppm, while it increased by 1.62% due to 700 ppm over aCO_2 . The AbSL of Harsha showed similar changes and decreased by around 16% in both the elevated CO_2 conditions. The AbSL of Varun decreased by 15.46% and 3.87% due to 550 ppm and 700 ppm over aCO_2 , respectively.

The AbSL in DHM-117 increased by 3.97% due to 550 ppm and decreased by 18.86% due to 700 ppm.

Physiological parameters

The performance of different physiological parameters of four maize genotypes – Harsha, Varun, DHM-117 and M-24 was quantified at aCO_2 and eCO_2 conditions of 550 ppm and 700 ppm. The parameters recorded are photosynthetic rate (P_n), stomatal conductance, transpiration rate, and water use efficiency. The physiological parameters of maize genotypes varied significantly under eCO_2 . The ANOVA revealed that genotypes and CO_2 levels highly significantly ($P < 0.01$) varied, and the interaction of $G \times C$ was highly significant ($P < 0.01$) for g_s and significant ($P < 0.05$) for P_n , T_r and WUE (Table 1).

Photosynthetic rate. As proved by various studies, in this experiment, the P_n of all four genotypes increased at eCO_2 compared to aCO_2 . The P_n of M-24 was 42.9, 45.3 and 45.4 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ at aCO_2 , 550 ppm and 700 ppm, respectively. The P_n of Harsha was 40.5, 49.9 and 48.7 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ at aCO_2 , 550 ppm and 700 ppm, respectively. The P_n of Varun was 41, 42.6 and 42.4 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ at aCO_2 , 550 ppm and 700 ppm, respectively, while the P_n of DHM-117 was 43.3, 49 and 50.1 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ at aCO_2 , 550 ppm and 700 ppm, respectively. The P_n of M-24 has increased by 5.52% and 5.79% at 550 ppm and 700 ppm, respectively, over aCO_2 (Table 3).

Stomatal conductance. The stomatal conductance was decreased in all four genotypes under both

Table 3. Mean *per se* values of different physiological parameters of four maize genotypes under three CO_2 concentrations

Physiological parameter	M-24			Harsha			Varun			DHM-117		
	ambient	550	700	ambient	550	700	ambient	550	700	ambient	550	700
		(ppm)			(ppm)			(ppm)			(ppm)	
P_n	42.9 ± 1.43	45.3 ± 1.42	45.4 ± 1.13	40.5 ± 1.17	49.9 ± 1.5	48.7 ± 2.41	41 ± 2.28	42.6 ± 1.25	42.4 ± 0.87	43.3 ± 1.05	49 ± 1.38	50.1 ± 1.79
g_s	0.43 ± 0.013	0.19 ± 0.009	0.18 ± 0.003	0.416 ± 0.14	0.26 ± 0.015	0.24 ± 0.014	0.27 ± 0.019	0.19 ± 0.019	0.15 ± 0.008	0.33 ± 0.02	0.22 ± 0.11	0.21 ± 0.019
T_r	9.42 ± 0.46	5.62 ± 0.22	6.06 ± 0.35	9.23 ± 0.32	7.04 ± 0.19	7.14 ± 0.39	7.56 ± 0.22	5.83 ± 0.24	5.63 ± 0.23	8.57 ± 0.26	6.21 ± 0.22	7.02 ± 0.43
WUE	4.59 ± 0.2	8.07 ± 0.13	7.56 ± 0.3	4.40 ± 0.14	7.09 ± 0.21	6.83 ± 0.13	5.42 ± 0.22	7.33 ± 0.21	7.58 ± 0.3	5.07 ± 0.24	7.91 ± 0.25	7.18 ± 0.23

P_n – photosynthetic rate ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$); g_s – stomatal conductance (cm/s); T_r – transpiration rate ($\text{mmol/m}^2/\text{s}$); WUE – water use efficiency ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)

eCO₂ levels compared to CO₂-grown plants. The g_s of M-24 were reduced by 56.15% and 57.73% under 550 ppm and 700 ppm, respectively, over aCO₂. The g_s of Harsha were reduced by 38.09% and 41.5% under 550 ppm and 700 ppm, respectively, over aCO₂. The g_s of Varun were reduced by 27.93% while 42.66% under 550 ppm and 700 ppm, respectively, over aCO₂. The g_s of DHM-117 were reduced by 33.15% while 35.19% under 550 ppm and 700 ppm, respectively, over aCO₂.

Transpiration rate. The transpiration rate was decreased in all four maize genotypes under both eCO₂ levels compared to aCO₂-grown plants. In M-24, the decrease in T_r due to eCO₂ over aCO₂ was 40.35% and 35.7% under 550 ppm and 700 ppm, respectively. The T_r of Harsha was 9.23, 7.04 and 7.14 mmol/m²/s under aCO₂, 550 ppm and 700 ppm, respectively. The T_r of the Varun genotype was decreased due to eCO₂ over aCO₂ by 22.86% and 25.61% under 550 ppm and 700 ppm, respectively, while T_r of DHM-117 decreased by 27.55% and 18.1% at 550 ppm and 700 ppm, respectively.

Water use efficiency. The water use efficiency increased in all four genotypes under both eCO₂ levels compared to aCO₂-grown plants. The WUE of M-24 was 4.59, 8.07 and 7.56 μmol CO₂/mmol H₂O under aCO₂, 550 ppm and 700 ppm, respectively. In Harsha, the increase in WUE was 61.05% and 55.06% under 550 ppm and 700 ppm, respectively, over aCO₂. The WUE of Varun was 5.42, 7.33 and 7.58 μmol CO₂/mmol H₂O under aCO₂, 550 ppm and 700 ppm, respectively. Meanwhile, in DHM-117, the increase in WUE was 55.81% and 41.54% under 550 ppm and 700 ppm, respectively, over aCO₂.

Biomass and yield parameters

At the final harvest, the mean performance of yield-related parameters – cob weight, grain weight, grain number, total biomass, harvest index and test weight of Harsha, Varun, M-24 and DHM-117 – was recorded under ambient control, 550 ppm and 700 ppm of CO₂.

The results of ANOVA suggest that there were highly significant ($P < 0.01$) differences observed between the four genotypes and CO₂ levels for cob weight, total biomass, grain yield, grain number, harvest index and test weight. The interaction of genotype and CO₂ levels registered a highly significant ($P < 0.01$) difference for seed number. In contrast, significant ($P < 0.05$) differences were observed with total biomass, grain weight and harvest index and non-significant for cob weight and test weight.

Cob weight. At the time of final harvest, the cob weight of Harsha was 117.47, 155.27 and 131.63 g/cob under aCO₂, 550 ppm and 700 ppm, respectively. The cob weight of Varun was 57.57, 75.0 and 57.83 g/cob under aCO₂, 550 ppm and 700 ppm, respectively. The cob weight of DHM-117 under aCO₂ was 122.8 g/cob; under 550 ppm, it was 175.63 g/cob; and under 700 ppm, it was 158.33 g/cob, while the cob weight of genotype M-24 was 116.03 g/cob, 129.87 g/cob and 118.23 g/cob under aCO₂, 550 ppm and 700 ppm, respectively. The increase in cob weight due to 550 ppm was 32.2, 30.3, 43 and 11.9% in Harsha, Varun, DHM-117 and M-24, respectively, while the increase in cob weight due to 700 ppm was 12.1, 0.5, 28.9 and 1.9% in Harsha, Varun, DHM-117 and M-24, respectively.

Grain yield. At the time of final harvest, the grain yield of genotype Harsha was 80.07 g/pl, 131.63 g/pl and 95.25 g/pl under aCO₂, 550 ppm and 700 ppm, respectively. The grain yield of Varun under aCO₂ was 46.33 g/pl; under 550 ppm, it was 64.15 g/pl, while under 700 ppm, it was 50.5 g/pl. The grain yield of DHM-117 was 80.03 g/pl, 117.83 g/pl and 91.8 g/pl under aCO₂, 550 ppm and 700 ppm, respectively, while the grain yield of genotype M-24 under aCO₂ was 72.73 g/pl, under 550 ppm it was 94.19 g/pl and under 700 ppm it was 81.68 g/pl. The increase in grain yield due to 550 ppm was 63.1, 38.4, 47.2 and 29.5% in Harsha, Varun, DHM-117 and M-24, respectively, while the increase in grain yield due to 700 ppm was 18, 9, 14.7 and 12.3% in Harsha, Varun, DHM-117 and M-24, respectively.

Grain number. At the time of final harvest, the grain number of Harsha under aCO₂ was 334.33/pl; under 550 ppm, it was 425.0/pl, while under 700 ppm, it was 374.67/pl. The grain number of Varun was 322.33/pl, 364.33/pl and 326.67/pl under aCO₂, 550 ppm and 700 ppm, respectively. The total number of a grain of DHM-117 under aCO₂ was 384.33/pl; under 550 ppm, it was 529.67/pl and under 700 ppm, it was 435.33/pl, while the grain number of genotype M-24 was 354.87/pl, 406.91/pl and 377.67/pl under aCO₂, 550 ppm and 700 ppm, respectively. The increase in grain number due to 550 ppm was 27.1, 13, 37.8 and 14.7% in Harsha, Varun, DHM-117 and M-24, respectively, while the increase in grain yield due to 700 ppm was 12.1, 1.3, 13.3 and 6.4% in Harsha, Varun, DHM-117 and M-24, respectively.

Harvest index. At the time of the final harvest, the harvest index of genotype Harsha under aCO₂ was 27.2%; under 550 ppm, it was 33.96%, while under

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700 ppm, it was 27.23%. The harvest index of Varun was 18.04, 21.35 and 18.88% under aCO₂, 550 ppm and 700 ppm, respectively. The harvest index of DHM-117 under aCO₂ was 21.09%; under 550 ppm, it was 24.24%; and under 700 ppm, it was 20.27%, while the harvest index of genotype M-24 was 19.87, 22.66 and 21.8% under aCO₂, 550 ppm and 700 ppm, respectively. The increase in harvest index due to 550 ppm was 24.9, 18.4, 15 and 14.1% in Harsha, Varun, DHM-117 and M-24, respectively, while the increase in harvest index due to 700 ppm was 0.1, 4.7, and 9.8% in Harsha, Varun, and M-24, respectively. A decrease of 3.9% was observed in DHM-117 for harvest index at 700 ppm over ambient CO₂.

Test weight. At the time of final harvest, the test weight of genotype Harsha under aCO₂ was 24.19, 30.96 and 25.43 g under aCO₂, 550 ppm and 700 ppm, respectively. The test weight of Varun under aCO₂ was 14.4 g; under 550 ppm, it was 17.61 g, while under 700 ppm, it was 15.45 g. The test weight of DHM-117 was 20.89, 22.22 and 21.11 g under aCO₂, 550 ppm and 700 ppm, respectively, while the test weight of genotype M-24 under aCO₂ was 20.49 g, under 550 ppm, it was 23.15 g, while under 700 ppm it was 21.63 g. The increase in the test weight due to 550 ppm was 28, 22.3, 6.4 and 13% in Harsha, Varun, DHM-117 and M-24, respectively, while the increase in test weight due to 700 ppm was 5.1, 7.3, 1 and 5.6% in Harsha, Varun, DHM-117 and M-24, respectively.

Biomass allocation. Biomass allocation to different plant parts differed under ambient, 550 ppm and 700 ppm CO₂ conditions. In Harsha, the biomass allocation at ambient CO₂ condition was 2.38, 17.3, 40.64 and 36.68% for root, leaf, stem and cob, respectively, 2.74, 15.29, 41.91 and 40.06% at 550 ppm, while 2.73, 16.5, 43.58 and 37.23% at 700 ppm, respectively. In Varun, the biomass allocation at ambient CO₂ condition was 5.7, 10.58, 61.33 and 22.39% for root, leaf, stem and cob, respectively, 5.85, 11.43, 57.78 and 24.93% at 550 ppm, while 5.5, 12.46, 60.43 and 21.6% at 700 ppm, respectively. In DHM-117, the biomass allocation at ambient CO₂ condition was 3.13, 19.73, 44.84 and 32.3% for root, leaf, stem and cob, respectively, 2.92, 16.73, 44.25 and 36.1% at 550 ppm, while it was 2.25, 17.38, 45.41 and 34.97% at 700 ppm, respectively. In M-24, the biomass allocation at ambient CO₂ condition was 3.01, 24.32, 41.02 and 31.65% for root, leaf, stem and cob, respectively; it was 3.21, 25.15, 40.41 and 31.23% at 500 ppm, while it was 3.31, 24.25, 40.92 and 31.52% at 700 ppm, respectively.

Correlation among different plant characters.

Table 4 presents a correlation coefficient analysis of the stomatal and physiological parameters of maize plants grown under ambient and elevated CO₂ conditions. The correlation between stomatal and physiological parameters of maize genotypes varied at different CO₂ concentrations.

Stomatal parameters. Under ambient CO₂ conditions, the TSD was highly significantly correlated ($P < 0.01$) with AdSD and AbSD only. While under 550 ppm, TSD was positively and significantly ($P < 0.05$) correlated with AbSL and highly significantly correlated ($P < 0.01$) with AdSD. At 700 ppm, TSD positively and significantly ($P < 0.05$) correlated with AbSD only.

Under ambient conditions, the AdSD was positively and significantly ($P < 0.05$) correlated with AbSD and AbSW while highly significantly correlated ($P < 0.01$) with TSD. While under 550 ppm, AdSD was positively and significantly ($P < 0.05$) correlated with AbSL and highly significantly correlated ($P < 0.01$) with TSD. At 700 ppm, none of the other stomatal parameters were correlated significantly.

Under ambient conditions, the AbSD was positively and significantly ($P < 0.05$) correlated with AdSD while highly significantly correlated ($P < 0.01$) with TSD. While less than 550 ppm, none of the other stomatal parameters were correlated significantly. At 700 ppm, AbSD was positively and significantly ($P < 0.05$) correlated with TSD, while negatively and significantly ($P < 0.05$) correlated with AbSL.

Under ambient conditions, the AdSW was positively and highly significantly correlated ($P < 0.01$) with AbSW, AdSL and AbSL. While under 550 ppm, AdSW was positively and significantly ($P < 0.05$) correlated only with AdSL. At 700 ppm, none of the other stomatal parameters were correlated significantly.

Under ambient conditions, the AbSW was positively and highly significantly correlated ($P < 0.01$) with AdSW, AdSL and AbSL. While under 550 ppm, AbSW was positively and significantly ($P < 0.05$) correlated only with AdSL. At 700 ppm, none of the other stomatal parameters were correlated significantly.

Under ambient conditions, the AdSL was positively and highly significantly correlated ($P < 0.01$) with AdSW, AbSW and AbSL. While under 550 ppm, AdSL was positively and significantly ($P < 0.05$) correlated only with AdSW and AbSW. At 700 ppm, none of the other stomatal parameters were correlated significantly.

Under ambient conditions, the AbSL was positively and highly significantly correlated ($P < 0.01$) with

Table 4. Correlation of stomatal and physiological parameters under three CO₂ concentrations

	P _n	g _s	T _r	WUE	AdSD	AbSD	TSD	AdSW	AbSW	AdSL	AbSL
Ambient											
P _n		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
g _s			**	**(-ve)	ns	ns	ns	**	*	**	*
T _r				**(-ve)	ns	ns	ns	**	*	**	**
WUE					ns	ns	ns	*(-ve)	ns	*(-ve)	*(-ve)
AdSD						*	**	ns	*	ns	ns
AbSD							**	ns	ns	ns	ns
TSD								ns	ns	ns	ns
AdSW									**	**	**
AbSW										**	**
AdSL											**
AbSL											
550 ppm											
P _n		*	ns	ns	ns	**(-ve)	ns	ns	ns	ns	ns
g _s			**	ns	ns	*(-ve)	ns	ns	ns	ns	ns
T _r				ns	ns	*(-ve)	ns	ns	ns	ns	ns
WUE					*	ns	*	ns	ns	ns	ns
AdSD						ns	**	ns	ns	ns	*
AbSD							ns	ns	ns	ns	ns
TSD								ns	ns	ns	*
AdSW									ns	*	ns
AbSW										*	ns
AdSL											ns
AbSL											
700 ppm											
P _n		*	**	ns	ns	ns	**	ns	ns	ns	ns
g _s			**	**(-ve)	ns	ns	*	ns	ns	ns	ns
T _r				**(-ve)	*	ns	ns	ns	ns	ns	ns
WUE					*(-ve)	ns	*(-ve)	ns	ns	*	ns
AdSD						ns	ns	ns	ns	ns	ns
AbSD							*	ns	ns	ns	*(-ve)
TSD								ns	ns	ns	ns
AdSW									ns	ns	ns
AbSW										ns	ns
AdSL											ns
AbSL											

* $P < 0.05$; ** $P < 0.01$; ns – non significant; P_n – photosynthetic rate; g_s – stomatal conductance; T_r – transpiration rate; WUE – water use efficiency; AdSD – adaxial stomata density; AbSD – abaxial stomata density; TSD – total stomatal density; AdSW – adaxial stomata width; AdSL – adaxial stomata length; AbSW – abaxial stomata width; AbSL – abaxial stomata length

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AdSW, AbSW and AdSL. While under 550 ppm, AbSL was positively and significantly ($P < 0.05$) correlated only with AdSD and TSD. At 700 ppm, AbSL was negatively and significantly ($P < 0.05$) correlated only with AbSD.

Physiological parameters. The correlation with-in physiological parameters differed significantly at each level of CO₂ concentration. Under ambient CO₂ conditions, P_n was not correlated with any other physiological parameter, whereas at 550 ppm, it had a positive significant ($P < 0.05$) correlation with g_s , while at 700 ppm, a positive significant ($P < 0.05$) correlation with g_s and positive and highly significant ($P < 0.01$) correlation with T_r . The g_s showed a positive and significant ($P < 0.01$) correlation with T_r at all three CO₂ concentration conditions. Under ambient CO₂ and at 700 ppm, WUE was negatively and significantly ($P < 0.01$) correlated with g_s and T_r , while at 550 ppm, no significant correlation was observed.

Stomatal and physiological parameters. Under ambient conditions, it was found that TSD was not correlated with any physiological parameter, while at 550 ppm, it was correlated positively and significantly ($P < 0.05$) with WUE. At 700 ppm, TSD was positively and highly significantly ($P < 0.01$) correlated with P_n , significantly ($P < 0.05$) with g_s and significantly ($P < 0.05$) and negatively correlated with WUE.

AdSD was positively and significantly ($P < 0.05$) correlated with WUE at 550 ppm and 700 ppm; it was positively and significantly ($P < 0.05$) correlated with T_r and significantly ($P < 0.05$) and negatively correlated with WUE at 700 ppm.

AbSD is correlated significantly ($P < 0.05$) and negatively with P_n , g_s and T_r only at 550 ppm.

AdSW was positively and highly significantly ($P < 0.01$) correlated with g_s and T_r significantly ($P < 0.05$) and negatively correlated with WUE. Its correlation with physiological parameters at 550 ppm and 700 ppm is non-significant.

AbSW was positively and significantly ($P < 0.05$) correlated with g_s and T_r , and its correlation with physiological parameters at 550 ppm and 700 ppm is non-significant.

AdSL was positively and highly significantly ($P < 0.01$) correlated with g_s and T_r , significantly ($P < 0.05$) and negatively correlated with WUE. Its correlation with physiological parameters at 550 ppm was non-significant. At 700 ppm, AdSL was positively and significantly ($P < 0.05$) correlated with WUE.

AbSL was positively and highly significantly ($P < 0.01$) correlated with T_r , significantly ($P < 0.05$) and positively with g_s , while negatively correlated with WUE and its correlation with physiological parameters at 550 ppm and 700 ppm are non-significant.

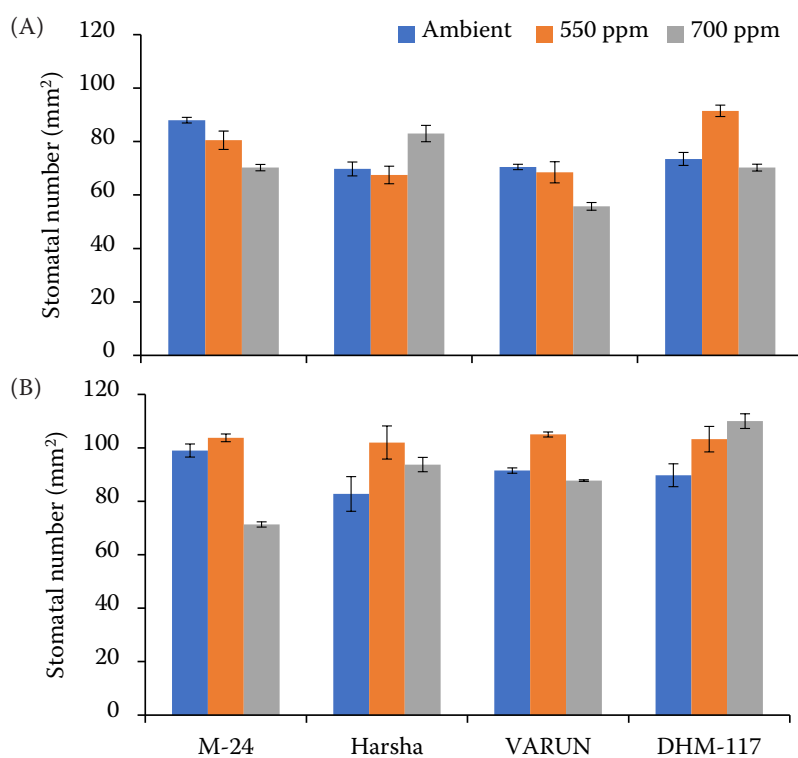


Figure 1. Stomatal density of (A) adaxial and (B) abaxial leaf surfaces of four maize genotypes (M-24, Harsha, Varun and DHM-117) at ambient, 550 ppm and 700 ppm CO₂ concentration

DISCUSSION

In terms of stomatal metrics, it was found that in both ambient ($a\text{CO}_2$) and elevated CO_2 ($e\text{CO}_2$) circumstances, all maize genotypes had a higher stomata density on the abaxial than the adaxial surface (Figure 1). Zheng et al. (2013) also observed that, when cultivated in ambient circumstances, maize leaves had more stomata on their abaxial than adaxial surfaces. The amount of CO_2 in the atmosphere (Woodwar 1987), light (Gay and Hurd 1975), and other environmental conditions all affect stomatal density.

Among the selected four maize genotypes, under ambient conditions, the highest adaxial surface stomatal density and abaxial surface stomatal density were recorded with M-24. Here, the number of stomata on the abaxial surface in ambient conditions was higher by 12.5% than the adaxial, and it further increased to 550 ppm as there was an increase in AbSD. However, at 700 ppm, the stomatal number on both surfaces decreased significantly. The response of Harsha was also similar, with a higher increase of abaxial stomatal number at 550 ppm and reduced at 700 ppm. In Varun, there is a linear increase in AbSD and a decrease in AdSD with 550 ppm and 700 ppm. In DHM-117, it was interesting to observe that at 550 ppm, the increase in AdSD was more than AbSD, while at 700 ppm, there was a decrease in AdSD and an increase in AbSD.

At 550 ppm, DHM-117 had the highest stomatal density on the adaxial surface, and Varun had the highest on the abaxial surface. At 700 ppm, Harsha and DHM-117 had the highest AdSD and AbSD, respectively. This demonstrates unequivocally that as CO_2 concentration increased from ambient to 550 ppm and 700 ppm, the stomatal density of maize genotypes varied dramatically. As CO_2 levels rose, M-24's stomatal density fell, reaching its lowest point at 700 ppm. Along with a slight rise at 550 ppm, the genotype Varun also showed a lower stomatal density at 700 ppm than ambient. At 550 ppm and 700 ppm, respectively, Harsha and DHM-117 recorded higher stomatal densities than the ambient conditions.

In general, the influence of 550 ppm was greater on AbSD than AdSD, while at 700 ppm, both AbSD and AdSD were reduced in maize. The response trend of the selected four genotypes differed at individual CO_2 levels and for their AbSD and AdSD. This was also reflected in the Ad/Ab ratio at all three CO_2 levels, as M-24, Harsha, and Varun recorded increased AbSD

and decreased AdSD at 550 ppm, which reduced the Ad/Ab ratio compared to ambient. At 700 ppm, AdSD was lower than ambient in M-24, Varun and DHM-117, while AbSD was lower in M-24 and Varun. In Harsha, though AdSD decreased from ambient to 550 ppm and 700 ppm, the AbSD increased at 550 ppm and decreased at 700 ppm. The impact of elevated CO_2 on AbSD and AdSD is genotype-specific, and it also differs with adaxial and abaxial surfaces. Under elevated CO_2 , *Pinus sylvestris* L. responded differently on both adaxial and abaxial surfaces in terms of their stomatal density (Lin et al. 2001), and variations were also found in this study.

The total stomatal density among the four maize genotypes was highest with M-24 under ambient conditions; however, the impact of elevated CO_2 of 550 ppm was non-significant in M-24 while DHM-117 recorded maximum increment followed by Harsha and Varun for TSD. It was interesting to note that at 700 ppm, Harsha recorded a further increment in TSD, while the other three genotypes recorded a decrease in TSD. It was evident that the increment in TSD in Harsha was mainly due to the increase in AbSD. Although showing a decrease in stomatal density with an increase in atmospheric CO_2 is common, it is not the same for all species (Drake et al. 1997). Seven of eight species studied on the relationship between stomatal characters and CO_2 found that mean stomatal density tended to be higher at elevated CO_2 (Reid et al. 2003). Studies with C_4 grass by Knapp et al. (1994) showed that most stomata were on the abaxial leaf surfaces, and the ratio of adaxial to abaxial stomatal density was greater at elevated levels of CO_2 . Maroco et al. (1999) found that leaves of CO_2 -enriched maize plants contained approximately 10% fewer stomata per unit leaf area than leaves of control plants. Species responded from pre-industrial to present CO_2 concentrations by changing stomatal size and number rather than solely by stomatal density (Maherali et al. 2002). In response to climate warming, plants alter stomatal frequency and change stomatal aperture size anatomically (Zheng et al. 2013). The present study found that the impact of $e\text{CO}_2$ on stomatal width and length was observed with all the maize genotypes; however, the individual genotype response differed (Figure 2). At ambient CO_2 conditions, stomatal width at adaxial surface (AdSW) ranged from 54.96 μm (M-24) to 39.0 μm (Varun) and at 550 ppm, it decreased in DHM-117 and Harsha, while increased with Varun and M-24 than ambient (Table 2). At 700 ppm, the AdSW decreased in M-24 and Harsha, while an increase of 21% in both Varun and DHM-117 was recorded.

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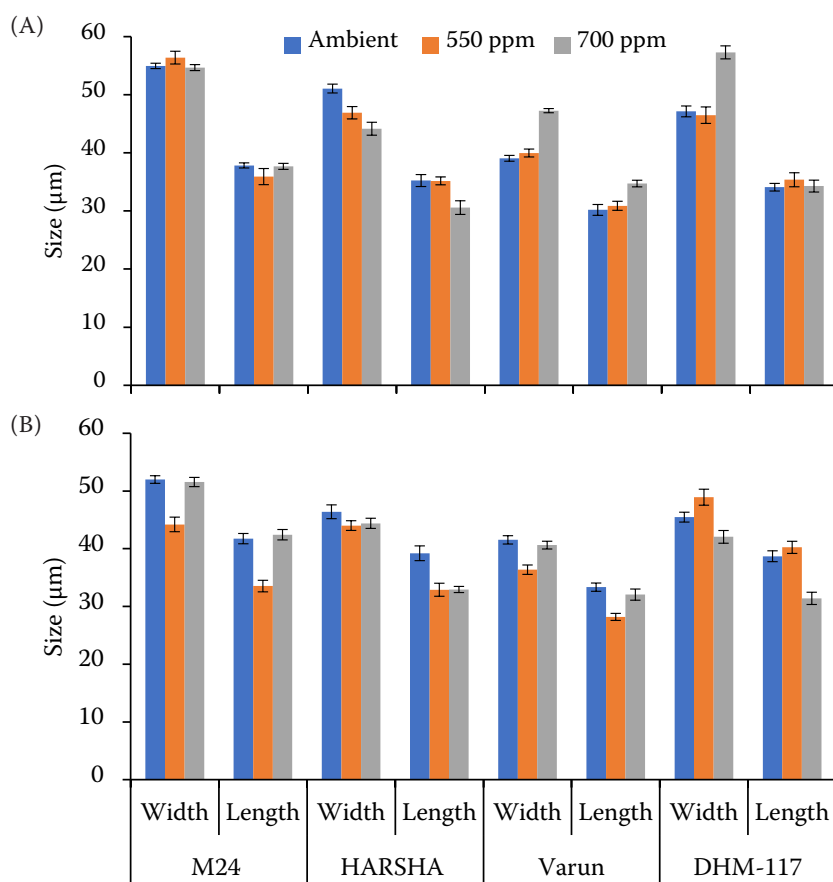


Figure 2. Stomatal width and length on (A) adaxial and (B) abaxial leaf surfaces of four maize genotypes (M-24, Harsha, Varun and DHM-117) at ambient, 550 ppm and 700 ppm CO₂ concentration

The stomatal width at the abaxial surface varied at ambient conditions, ranging from 51.98 μm (M-24) to 41.53 μm (Varun). In Harsha, Varun, and M-24, it reduced at 550 ppm, whereas in DHM-117, it grew. At 700 ppm, the AbSW dropped in each of the four genotypes to varying degrees.

At ambient conditions, the stomatal length at the adaxial surface varied from 37.83 μm (M-24) to 30.18 μm (Varun). It grew in DHM-117, Varun and decreased in M-24 (5%), whereas Harsha showed no change in this parameter. Similarly, at 700 ppm, it went up in Varun and down in Harsha, but there was very little change in M-24 and DHM-117 (Table 2).

The stomatal length at the abaxial surface under ambient conditions ranged from 41.75 μm (M-24) to 33.34 μm (Varun), and it decreased at both 550 ppm and 700 ppm in Harsha and Varun, while in M-24, it decreased at 550 ppm and increased at 700 ppm. The reverse trend of response was recorded with DHM-117 as AbSL increased at 550 ppm (3.97%) and decreased at 700 ppm (18.86%).

From these results, it can be inferred that the maize genotypes differed significantly in density and size of stomata at both elevated CO₂ levels. The genotype

Harsha registered an increase in density and decrease in size of stomata in both elevated levels of CO₂. The response of M-24 was different under both treatments as the change in stomatal density was non-significant at 550 ppm but significant at 700 ppm, while a high decrease in stomatal size was recorded by both M-24 and Varun at 550 ppm. In DHM-117, stomata density increased at both CO₂ levels while the size increased at 550 ppm and decreased at 700 ppm. The response of this genotype's adaxial and abaxial SD and stomatal size also varied with CO₂ concentrations.

The physiological characteristics of the several genotypes of maize under eCO₂ varied significantly in terms of photosynthetic rate, stomatal conductance, transpiration rate, and water usage efficiency. All of the genotypes of maize exhibited reduced rates of transpiration and stomatal conductance as well as higher rates of photosynthetic efficiency and WUE under situations with elevated CO₂ levels (Table 3). Maize demonstrated increased CO₂ absorption at rising CO₂ conditions, caused by lower T_r and higher intracellular CO₂ content at low g_s (Bunce 2014).

In this study, all the maize genotypes recorded an increased rate of photosynthesis at higher CO₂

conditions of 550 ppm, and this changed pattern was also maintained under 700 ppm. Plants exposed to elevated CO₂ often show increased growth and water use efficiency (Rogers and Dahlman 1993) and increased rates of photosynthesis (Amthor 1995, Linke et al. 2022). Leakey et al. (2004) reported that elevated CO₂ significantly increased leaf photosynthetic rate in maize plants, around 41% higher than ambient control. The higher photosynthetic rates of plants raised under elevated CO₂ may be due to the high availability of CO₂ as a substrate for carboxylation but can also be related to the activation state of Rubisco for carbon fixation (Habash 1995, Xu et al. 2022).

Stomatal conductance was decreased in all four maize genotypes under elevated CO₂ conditions. A maximum decrease in g_s was observed in M-24, where the g_s reduced by 56.15% at 550 ppm and 57.73% at 700 ppm. The genotypes Harsha, Varun and DHM-117 recorded linear decrease with an increase in CO₂ concentration as higher reduction was observed at 700 ppm (41.5, 42.66, and 35.19%) than at 550 ppm (38.09, 27.93, and 33.15%). Among the genotypes, the decrease in g_s from 550 ppm to 700 ppm was significantly high in Varun, while the other three genotypes also recorded a decrease; however, it was non-significant. Increased CO₂ concentrations cause smaller stomatal apertures and lower the leaf conductance for water vapours (Morison 1987). Many species showed decreased stomatal conductance in response to increased CO₂ despite the lack of a decrease in stomatal density or stomatal index (Nowak et al. 2001). In creeping bentgrass (*Agrostis stolonifera* L.), the leaf T and g_s were reduced by 40% due to the elevated CO₂ treatment (Burgess and Huang 2014).

The transpiration rate in all the maize genotypes decreased with eCO₂. Under elevated CO₂, transpirational water loss reduces with a decrease in stomatal density of the leaf which occurs chiefly through an irreversible decline in maximum stomatal conductance (Eamus et al. 1993). In this experiment it was interesting to observe that the response of T_r to elevated CO₂ is not linear, as the decrease in T_r was significant at 550 ppm as compared with ambient and it maintained at same level with further increase in CO₂ concentration to 700 ppm. Among the genotypes, highest decrease in T_r was recorded with M-24 at both 500 ppm (40.35%) and 700 ppm (35.7%), while the magnitude of response was similar with Harsha, Varun and DHM-117 which ranged between 18.10% to 27.55%. Studies have shown that

elevated CO₂ reduced the leaves stomatal density, stomatal index, stomatal conductance and transpiration rate in *Arabidopsis thaliana* (L.) Heynh (Teng et al. 2006). In rice the elevated CO₂ resulted in stimulation of photosynthesis and down regulation of photosynthetic physiological parameters and stomatal area (Yang et al. 2023).

WUE commonly defined as the amount of carbon fixed in photosynthesis per unit of water transpired (Lawson and Blatt 2014). The WUE increased in all the maize genotypes in both eCO₂ treatments, and maximum increase was observed in M-24 at both higher CO₂ concentrations. The other three genotypes – Harsha, Varun and DHM-117 also showed high response to eCO₂ and the percentage increase was 61.05, 35.32 and 55.81 respectively at 550 ppm, while at 700 ppm it was 55.06, 39.93 and 41.54, respectively. The genotype response for photosynthetic rate and transpiration rate under eCO₂ was reflected into its WUE as the genotype M-24 showed a drastic reduction in T_r and therefore registered higher WUE. Harsha recorded higher P_n at both eCO₂ levels. With this data it is evident that response for T_r , g_s and WUE to elevated CO₂ was maximum in genotype M-24 while maximum response for P_n was observed with genotype Harsha. WUE of maize plants increased under enhanced CO₂ conditions due to improved P_n and reduced T_r (Khan et al. 2020). Leakey et al. (2004) proposed that CO₂-enriched maize plants showed 23% decrease in stomatal conductance which led to reduced transpiration rates. Another study did by Kim et al. (2006), revealed that maize plants grown at elevated CO₂ exhibited 4% increase in photosynthetic rates, 50% reductions in both leaf stomatal conductance and transpiration rate which resulted in doubling of leaf water use efficiency. Review studies done by Pospisilova and Catsky (1999) showed that in about 85% of the reported studies, elevated CO₂ increased net photosynthesis rates, while reduced stomatal conductance and rates of transpiration in approximately 75% of the cases analysed. Consequently, atmospheric CO₂ enrichment increased plant water use efficiency in more than 90% of the experiments that were conducted.

The increased CO₂ had a favourable effect on the plant height of the four genotypes of maize, as evidenced by the rise in stover biomass (Figure 3). In comparison to aCO₂, the greatest increase in plant height was observed in Harsha at 550 ppm, and the lowest at 700 ppm in DHM-117. According to Driscoll et al. (2006), maize plants can perform better

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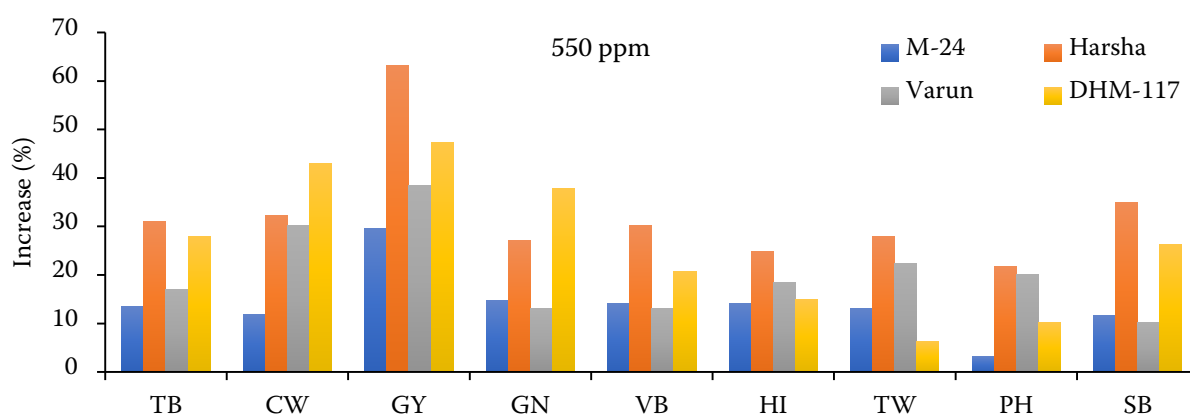


Figure 3. Impact of elevated CO₂ (550 ppm and 700 ppm) on biomass and yield parameters of four maize genotypes (M-24, Harsha, Varun and DHM-117). TB – total biomass; CW – cob dry weight; GY – grain yield; GN – grain number; VB – vegetative biomass; HI – harvest index; TW – test weight; PH – plant height; SB – stover biomass

in response to higher CO₂ concentrations because they are a C₄ crop. They observed a 23% increase in plant height at 700 ppm CO₂.

Under both elevated CO₂ conditions an increase was observed in all the biomass and yield contributing parameters of maize genotypes such as total biomass, cob dry weight, stover biomass, grain yield, number of grains per cob, 100 grain weight and harvest index. The increase in the yield parameters was more prominent at 550 ppm than at 700 ppm as compared to ambient CO₂ conditions (Figure 3). The total biomass of the four genotypes increased significantly under the eCO₂. At 550 ppm, increase in biomass ranged from 13.4% (M-24) to 30.9% (Harsha) while at 700 ppm it ranged from 2.3% (M-24) to 19.11% (DHM-117). Under elevated CO₂ conditions, it was interesting to observe that the increase in the reproductive biomass was higher than the vegetative biomass in Harsha, Varun and DHM-117. Khan et al. (2018) also reported that the contribution of reproductive biomass was more in increasing the total biomass in maize and groundnut under eCO₂, as compared to the vegetative biomass which was in lines to our findings. In this experiment it was found that maximum increase in the cob dry weight was observed in DHM-117 where at aCO₂ it was 122.8 g which increased to 175.6 g under 550 ppm and 158.3 g/cob at 700 ppm. Impact of high CO₂ on grain number was also more distinct at 550 ppm than at 700 ppm concentration, where DHM-117 recorded maximum increase, revealing that the biomass improvement was higher at 550 ppm than 700 ppm. The impact of elevated CO₂ on the grain yield was of considerable interest because a maximum of 63% increase was observed in Harsha

followed by 43% in DHM-117 and more than 30% in Varun and M-24. The increase in grain yield at 700 ppm ranged between 9% (Varun) to 18% (Harsha).

The reaction was greater at 550 ppm than at 700 ppm, and the HI rose as the eCO₂ level increased. Increased CO₂ considerably enhanced maize genotypes' HI (Vanaja et al. 2015). Our data clearly show that the increase in the reproductive portions of the C₄ maize was the primary factor in the enhancement of biomass in response to higher CO₂. Although possessing a C₄ photosynthetic pathway, maize was able to respond favourably to increased atmospheric CO₂ concentration, according to an analysis of genotypes of the crop conducted at elevated CO₂ (550 ppm) for biomass and yield (Vanaja et al. 2015).

Under ambient CO₂ conditions, it was observed that the total stomatal density was not correlated with any of the stomatal parameters while under 550 ppm, positive and significant correlation with AbSL ($P < 0.05$) and AdSD ($P < 0.01$) and also with AbSD at 700 ppm was observed. Majority of the studies reported that stomatal density is negatively correlated with stomatal size (Hetherington and Woodward 2003, Franks and Beerling 2009). However, our findings showed that the stomatal density on the adaxial and abaxial surface were positively and significantly correlated with AbSW and TSD under ambient CO₂ condition. At 550 ppm and 700 ppm also similar relations were maintained and these two parameters were significantly ($P < 0.01$) correlated with TSD and AbSL. At 700 ppm, the AbSL was in negative correlation with these two parameters.

In the presence of ambient CO₂, there was a substantial ($P < 0.05$) correlation between AdSW and AbSW, and between AbSW and AdSD. Under ambient

CO₂, there was a substantial ($P < 0.01$) correlation between the stomatal width on both surfaces (AdSW and AbSW) and the stomatal length on both surfaces (AdSL and AbSL). At 550 ppm, there was a substantial ($P < 0.05$) correlation between the stomatal width of both leaf surfaces and AdSL alone; however, at 700 ppm, no such correlation was seen. This study unequivocally shows that while changes in stomatal number at the adaxial surface at 550 ppm and the abaxial surface at 700 ppm were influencing the TSD, the number of stomata was not altering the size under ambient conditions. Additionally, it was noted that TSD at 550 ppm was affecting the leaf's abaxial surface stomatal length.

The correlation within physiological parameters differed significantly with concentration of CO₂. Under ambient CO₂ conditions, P_n was not correlated to any other physiological parameter whereas at both 550 ppm and 700 ppm it had positive significant ($P < 0.05$) correlation with g_s. Early experiments also illustrates that photosynthetic rates were correlated with stomatal conductance when other factors were not limiting (Wong et al. 1979). The g_s was showing positive and significant ($P < 0.01$) correlation with T_r at all the three CO₂ concentration conditions. Under ambient CO₂ and at 700 ppm, WUE was negatively and significantly ($P < 0.01$) correlated with g_s and T_r, while at 550 ppm no such correlation was observed. It was also found in previous studies that plants with higher g_s have greater assimilation rates and grow faster under optimal conditions, but they generally exhibit lower WUE which was also observed in our findings. Fischer et al. (1998) demonstrated a close correlation between g_s and yield in eight different wheat (*Triticum aestivum* L.) cultivars.

Under ambient control conditions it was found that TSD was not correlated to any physiological parameter while at 550 ppm it was correlated positively and significantly ($P < 0.05$) with WUE. At 700 ppm, TSD was positively and significantly ($P < 0.01$) correlated with P_n and T_r, and significantly ($P < 0.05$) with g_s. These are comparable with the findings of Tanaka et al. (2013) with *Arabidopsis* and Xu and Zhou (2008) in grass as the assimilation rate increased at elevated CO₂ was tightly associated with increased stomatal density. A negative association in bread wheat (*Triticum aestivum* L.) was observed between cell size and photosynthetic rate in wheat when grown in different seasons (Bhagwat et al. 1997). A positive correlation exists between P_n rates and stomatal frequency per unit leaf area in maize (Heichel 1971).

Several investigators have provided evidence that stomatal frequency and conductance or CO₂ uptake are correlated (Pallas 1980). Increase in stomatal density was positively and significantly ($P < 0.05$) correlated with WUE at 550 ppm and negatively at 700 ppm. It is clear from the data that with the increasing CO₂ levels, the relation in the anatomical and physiological parameters was changing. It is evident that increased stomatal number at 700 ppm improving P_n, g_s and T_r while reducing WUE while at 550 ppm it is improving WUE. This could be due to better improvement of P_n compared to reduction in T_r was contributed by increased stomatal number at 550 ppm than at 700 ppm.

Under control ambient conditions it was found that the correlation between g_s and length and width of stomata was highly significant ($P < 0.01$) for adaxial stomata, while significant ($P < 0.05$) for abaxial stomata, while such relation was absent at 550 ppm and 700 ppm of CO₂. Hetherington and Woodward (2003) and Franks and Beerling (2009) reported that under high CO₂ conditions, leaf maximum stomatal conductance is dependent on stomatal aperture size and shape, frequency, and distribution pattern. While it was also reported that leaf net assimilation rate was negatively correlated with stomatal density when plants were exposed to elevated CO₂ (Woodward 1987, Ainsworth and Rogers 2007). In the present study it was found that under ambient CO₂ conditions, stomatal density on both the leaf surfaces were not related with any physiological parameter. While at 550 ppm, AdSD was correlated significantly ($P < 0.05$) and positively with WUE while AbSD was correlated negatively and significantly with P_n, g_s and T_r. At 700 ppm, AdSD was correlated positively and significantly ($P < 0.05$) with T_r while negatively with WUE.

Under ambient control condition, stomatal width and length of both leaf surfaces was correlated significantly and positively with T_r and g_s while WUE was negatively and significantly correlated with stomatal width and length of adaxial leaf surface whereas positive correlation was observed with length of abaxial leaf surface. It was interesting to record that the stomatal size was not correlated with any physiological parameters at 550 ppm and only AdSL was correlated with WUE positively and significantly ($P < 0.05$) at 700 ppm. The literature already published reveal that leaf anatomy-specifically, the internal cell surface area per unit leaf area is highly correlated with photosynthesis and WUE (Sinclair et al. 1977). The responses of plants to large and rapid environmental changes within

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a generation cannot predict the long-term response of plants to natural environmental changes over multiple generations, especially in annual herbs with short life cycles (Yang et al. 2023). Under elevated CO₂ conditions it was seen that different maize cultivars were responding differently for stomatal, biomass, yield and physiological parameters. The total density of stomata increased with elevated CO₂ in three maize genotypes and it was mainly due to increased stomatal density on abaxial surface. The response of stomatal size was genotype and CO₂ concentration specific. Elevated CO₂ conditions increased P_n and WUE and decreased g_s and T_r in all the genotypes with different magnitude. The correlation analysis revealed that increase in stomatal density was positively and significantly correlated with Anet, g_s and T_r at 700 ppm while with WUE at 550 ppm. The increased stomatal density on abaxial surface contributed more in improved Anet at 550 ppm and improved WUE while at 700 ppm it also increased T_r hence reduced WUE. This was evidently reflected in the higher response of biomass and yield of maize genotypes at 550 ppm than 700 ppm.

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