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Evidence from simulated climatic conditions indicates rising CO_2 levels impact pearl millet yield and nutritional traits

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ABSTRACT

Rising atmospheric CO_2 significantly impacts crop productivity and nutritional quality, posing challenges to global food security. Pearl millet (*Pennisetum glaucum* (L.) R. Br.), a climate resilient nutri-cereal, plays a vital role in food and nutrition security particularly in arid and semi-arid regions of India and sub-Saharan African countries. However, its response to changing climate conditions such as elevated CO_2 are not well known. This study assessed the response of various pearl millet genotypes, including hybrids and inbred lines to elevated CO_2 (550 and 700 ppm) from the current level of 420 ppm. Elevated CO_2 resulted in enhanced plant height, chlorophyll content, and nitrogen balance index. However, average grain yield recorded 1.2 % reduction at 550 ppm and 28.8 % at 700 ppm. Flavonoid concentration increased at 550 ppm (5.1 %) but decreased at 700 ppm (14.5 %). Average grain Fe and Zn content increased at 550 ppm by 4.25 % and 6.12 %, respectively but declined at 700 ppm by 4.01 % and 7.04 %; however in ICMB 92111, ICMB 92888, HHB67Imp and NBH 4903 increased Fe accumulation was recorded at 700 ppm) Grain protein content decreased significantly (1.12 % at 550 ppm, 13.4 % at 700 ppm), while fodder protein increased (16.01 % at 550 ppm, 15.19 % at 700 ppm). These findings highlight the complex effects of CO_2 fertilization on pearl millet's productivity and nutritional profile; the crop remains relatively resilient up to 500 ppm CO_2 but becomes more susceptible to negative impacts at 700 ppm. Therefore, large-scale germplasm evaluation and targeted breeding efforts are essential to develop climate-resilient genotypes with stable yields and enhanced nutrient content under future CO_2 conditions.

1. Introduction

The carbon dioxide (CO_2) concentration in the atmosphere have been steadily rising from 280 ppm in the pre-industrial era (1960s) to current levels of 420 ppm and is predicted to rise to as much as 700–1000 ppm by the end of this 21st century [1–3]. This increase is part of a broader pattern of climate change, driven largely by human activities such as the burning of fossil fuels, deforestation, and industrial processes [4,5]. Rising CO_2 can indeed enhance plant growth by increasing the availability of carbon for photosynthesis and changing plant physiology, however it alters essential elements such as protein, iron (Fe), and zinc (Zn) in many crop species [6,7]. In past few years, multiple studies and meta-analyses indicated that increasing CO_2 will reduce protein and mineral concentrations from a wide-variety of plant-based food sources including wheat, soybeans, and rice. This direct effect of rising CO_2 on the nutritional value of crops represents a potential threat to the nutritional security of billions of people and human health.

C_3 plants have a higher photosynthetic response to elevated CO_2 than C_4 and Crassulacean Acid Metabolism (CAM) plants, as C_3 plants use CO_2 directly in their carbon fixation while C_4 plants have a CO_2 concentrating mechanism that makes them less sensitive to changes in CO_2 concentration [8]. The essential difference between the C_3 and C_4 modes of photosynthesis is that CO_2 partial pressure at the site of

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Rubisco (a carbon-fixing enzyme) is 5–10 times higher in C_4 compared to C_3 photosynthesis. This effectively prevents photorespiration by suppressing O_2 competition. The photosynthetic enhancement of C_4 plants to increased CO_2 is about half that of C_3 plants [9]. Moreover, doubling the current ambient CO_2 concentration stimulates the growth of C_3 plants by 40–45 %, compared to 10–20 % in C_4 plants [10]. Further, the effects of CO_2 on plants can vary depending on the crop and other environmental factors. [11]. For instance, while CO_2 can increase the uptake of some mineral elements, it may also decrease the uptake of others, potentially altering the nutritional quality of the plants [12]. This can have significant implications for soil nutrient balance, carbon storage, and human nutrition, especially in terms of protein, vitamins and minerals [13].

The CO_2 related decrease in the nutritional quality of crops will exacerbate the global challenges of food and nutrition security. While many studies indicate a potential positive CO_2 fertilization effect with respect to biomass accumulation, particularly in C_3 crops [14,15], it is now well established that atmospheric enrichment in CO_2 has a negative impact on the nutritional quality content of staple crops [16]. Negative nutritional impacts include reduced protein content [11,17] and reductions in the accumulation of essential mineral nutrients in crop tissues [18,19]. It is estimated that elevated CO_2 could result in additional 175 million people becoming Zn deficient and 122 million becoming protein deficient while increased Fe deficiency risk could affect 1.4 billion people in Asia, Africa, and the Middle East [20]. Growing evidence suggests the declining nutritional quality of grain crops, especially cereals crops which provide billion of tons of proteins and nutrients globally for human consumption and livestock feed [21]. For example, a CO_2 mediated protein reduction of 10–15 % was reported in barley, rice, and wheat [22]; a reduction of Fe and Zn concentration was also reported in these crops [16]. In another study, a decrease of essential elements like nitrogen, phosphorus, potassium, and sulphur was observed in wheat, barley, potato and sorghum in the study conducted under specialized facilities such as free air CO_2 enrichment (FACE) and Open-top chamber (OTC) [23]. Any disruption in the availability or nutritional quality of these staples could significantly threaten human nutrition, especially in low-resource countries where people depend on cereal grains and legumes for their micronutrient intake and alternative sources of these micronutrients are scarce [24].

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), a staple food for over 90 million people, is a climate resilient C_4 cereal grown in the hot semi-arid tropics of Asia and Africa with low and erratic rainfall. An efficient CO_2 fixation, reduced photorespiration, higher water-use efficiency and greater tolerance to heat and drought underpin pearl millet superior performance in arid and semi-arid environments [25]. Pearl millet has been shown to have the highest Fe (18–121 mg/kg) and Zn (22–87 mg/kg) content among the major cereals such as rice, wheat, maize, and sorghum [26]. However, commercially cultivated pearl millet varieties and hybrids contain an average of 42 mg/kg of Fe and 32 mg/kg Zn in the grains [27–29]. Biofortification of pearl millet could play a significant role in combating micronutrient deficiencies that affect immune system functionality and pose a global health challenge, particularly in low-income countries where dietary diversity is limited [30]. Considering the existence of large variability for Fe and Zn density in pearl millet, significant breeding efforts were made to improve the levels of Fe and Zn content in pearl millet lines, cultivars, and the performance of hybrids [31].

Biofortified pearl millet varieties are gaining importance in the farming community and studies have shown the positive impact of its consumption [32,33]; therefore, information on the effect of CO_2 on pearl millet micronutrients (Fe and Zn) and protein concentration can guide and streamline future crop breeding efforts by revealing which germplasm/cultivars are more likely to provide more adequate nutrition under changing CO_2 levels. Thus, quantifying the effect of increasing CO_2 on human nutrition requires accounting for changes in the quantity and quality of crop harvests and human diets. This study was conducted to assess the impacts of increasing CO_2 (550 and 700 ppm) on pearl millet production parameters, yield, and flavonoids content in leaves; protein content in fodder and grain; and Fe and Zn content in grains under simulated conditions.

2. Materials and methods

2.1. Plant material and experimental design

The study involved ten pearl millet genotypes that represent diverse genetic material groups, including inbred lines (ICMB 98222, ICMB 1505, ICMR 12555, ICMB 92111, ICMB 92888) and hybrids (ICMH 1301, ICMH 1202, 86M86, HHB 67 Imp, NBH 4903) (Table 1). The experiment was conducted during the rainy season using a completely randomized design (CRD) with four replications. The seeds of these lines were produced and procured from the pearl millet breeding program at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India. Healthy seeds of these genotypes were sown in 12-inch plastic pots filled with red soil (pH 7.2; electrical conductivity 0.32 dS/m; organic matter 0.63 %) and in each pot three plants were grown and maintained. The OTC structure ensures each plant experiences similar growth conditions, allowing for accurate comparisons between different genotypes. Standard crop management practices were followed to ensure the plants were raised under optimal conditions until harvest.

2.2. Growth conditions

The study was conducted in the OTC at the Center of Excellence in Climate Change Research for Plant Production and Protection, ICRISAT. The OTCs (4.0 m L x 4.0 m W x 3.5 m H) are specialized facilities that allow a specific level of CO_2 concentration to be maintained through the emission of CO_2 from cylinders when needed (.). They are equipped with various remote controlling systems like an infrared heater module (T-FSR 1000, Elstein, Germany), ultrasonic humidifier (Genesis Technology, India), temperature sensors (HK Tempsensors, India), humidity sensors with transmitter (Rotronic, Switzerland), data scanners, and a CO_2 monitoring system with non-dispersive infrared absorption (NDIR) based CO_2 analyzer (Topac, USA); these systems are enabled with integrated system control (Winlog control software) (SCADA) to maintain the desired levels of temperature, CO_2, and humidity in the chambers.

Table 1
Details of the peal millet genotypes used in the study.

Sl. No.	Genotypes Identity	Breeding use	Genotypes features
1	ICMB 1505	Seed parent	ICRISAT seed parent with high Fe (115–125 ppm) and high Zn (45–50 ppm)
2	ICMB 98222	Seed parent	ICRISAT seed parent with high Fe (90–105 ppm) and Zn (40–45 ppm)
3	ICMB 92111	Seed parent	ICRISAT seed parent with low Fe (35–40 ppm) and low Zn (27–30 ppm)
4	ICMB 92888	Seed parent	ICRISAT seed parent with low Fe (38–42 ppm) and low Zn (27–33 ppm)
5	ICMR 12555	Restorer parent	ICRISAT restorer parent with high Fe (80–85 ppm) and Zn (35–40 ppm)
6	ICMH 1202	Hybrid	Public commercial hybrid with high Fe75-80 ppm) and Zn (35–40 ppm)
7	ICMH 1301	Hybrid	Public commercial hybrid with high Fe (75–92 ppm) and Zn (35–40 ppm)
8	HHB 67 Imp.	Hybrid	Public commercial hybrid with low Fe (55–60 ppm) and Zn (35–40 ppm) early maturity
9	86M86	Hybrid	Private commercial high-yielding hybrid with medium Fe (60–65 ppm) and Zn (35–40 ppm)
10	NBH 4903	Hybrid	Private commercial high-yielding hybrid with low Fe (52–57 ppm) and Zn (35–38 ppm)

For this study, three CO_2 variables were set at ambient CO_2 (~415 ppm), 550 ± 25 ppm and 700 ± 25 ppm. The pure CO_2 was pumped from cylinders with integrated system control (SCADA) through underground piping connected to each OTC at sonic speed to allow rapid mixing of CO_2 with air. The CO_2 was pumped only during the daytime from sowing to harvesting (Fig. 1). Data storage and backup were completed in a readily accessible format for real-time monitoring of each climatic factor [34].

2.3. Observations

Data was recorded on productivity parameters, morphological, and yield related traits such as plant height (cm), days to 50 % flowering, number of tillers, panicle length (cm), panicle diameter (mm), panicle weight (g), grain yield (g), and dry fodder weight (g) from each treatment. Further, grains were harvested from each treatment for protein, Fe, and Zn analysis. The grain samples were cleaned and allowed to sun dry with caution, being shielded from dust and metal contact contamination. Approximately 30 g grain samples were collected from each replication and stored in clean and non-metal folded paper bags at room temperature. A dried whole plant, upon maturation, was selected from each replication and chopped into small pieces before processing for analysis [35].

2.4. Estimation of total flavonoids, chlorophyll content, and NBI

Total flavonoids, chlorophyll content, and nitrogen balance index (NBI) were recorded 30 days after sowing using a phenol meter (Force A Paris, France). Total flavonoids and chlorophyll content were expressed as $\mu g\ cm^{-2}$ and NBI as the ratio of chlorophyll to flavonoids (Chl/Flav). A fully expanded leaf was placed between the two parts of the phenol meter; four observations were recorded from each plant and three plants for each treatment were taken into consideration with necessary calibrations of the instrument [36].

2.5. Determination of protein content in fodder and grains

The protein content in fodder and grain was analysed using near-infrared spectroscopy (NIRS). Before scanning, the samples were dried at 50 °C for 16 h and cooled to room temperature. The samples were then scanned using a NIR spectrometer DS2500 flour analyzer from FOSS (FOSS-DS2500; FOSS Electric A/S, Hillerød, Denmark). For obtaining the spectral sample signature from the FOSSDS2500, each sample was transferred to the standard circular ring cup (inside diameter ~6 cm, FOSS sample cup) and scanned three times at room temperature (~26 °C). The sample was mixed before each scan. The NIR spectral absorbance, with a range of 400–2498 nm, was recorded as the logarithm of reciprocal reflectance (1/R) with 2 nm intervals, using the Win ISI spectral analytical software (v4.4, Infra Soft International LLC, PA, USA). The quantified grain protein content was measured in percentage (%) [37].

2.6. Micronutrient analysis

The grain samples of all genotypes were frozen before nutrient analysis. The samples were homogenized to a fine powder. The powdered sample was sieved through a mesh and then dried completely for constant weight at 70 °C. For the grain mineral nutrients assay, 500 mg of powdered samples were added to the graphite tube for digestion, 0.2 ml of pure deionized (DI) water was added, followed by 8 ml of nitric acid (HNO_3) and 2 ml of 35 % hydrogen peroxide (H_2O_2) in a Teflon container, and samples were heated in a closed microwave digestion system, and digested for 24 h. To manage the heating gradient, digestion temperature was regulated for 220 °C for 20 min and 180 °C for 10 min, regulated until a clear-colored solution was obtained. Finally, the digested solution was diluted to 50 ml by adding deionized water. Inductively coupled plasma (ICP) Optical Emission Spectrometer (Thermo 7700, Agilent, Thermo Fisher Scientific, Waltham MA), hereafter referred to as ICP analysis, was used to determine Fe and Zn content. The inclusion of aluminium (Al) density estimation serves as an indicator for potential soil or dust contamination (Stangoulis 2017). Mineral concentrations were validated by interspersing experimental samples with standard reference materials of a known element. The quantified grain Fe and Zn levels were measured in milligrams per kilogram (mg/kg) of seed [38].

2.7. Statistical analysis

Statistical analysis and data visualizations were performed using the R statistical program [39]. The Shapiro-Wilk normality test was performed to determine the normal distribution of samples [40]. Individual and combined analysis of variance (ANOVA) was performed for protein, Fe, and Zn content to test the significance of genotypes, CO_2, and interaction of genotypes × CO_2. The percent changes over ambient at 700 and 550 ppm were calculated in MS Excel, and figures and Pearson's correlation coefficients were calculated using R statistical program [39].

3. Results

3.1. Effect of elevated CO_2 on pearl millet growth and yield related parameters

The ANOVA highlighted the significant differences among genotypes and elevated CO_2 on growth and yield related parameters (Table 2).

Plant height- Irrespective of genotypes, plant height increased significantly ($P < 0.01$) with CO_2, reflecting enhanced biomass

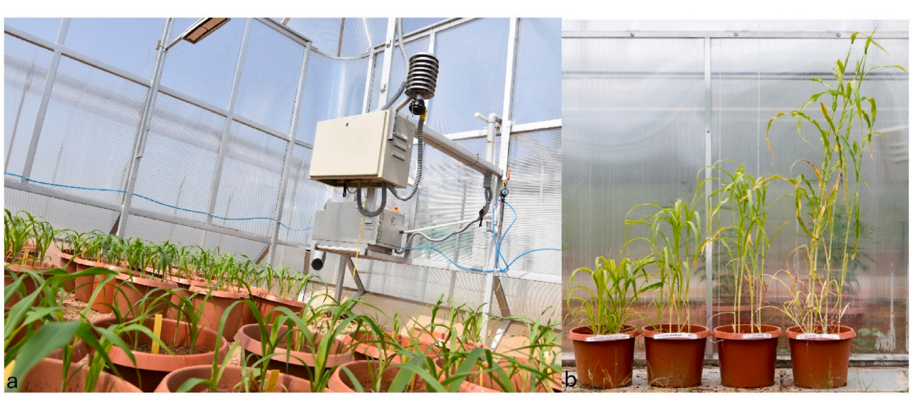

Fig. 1. The appearance of pearl millet grown under simulated CO_2 conditions in Open top chambers. (a) Plants growing inside the OTC; (b) comparison of plant height grown under different CO_2 level.

Table 2
Analysis of variance (ANOVA) for pearl millet genotypes response to elevated CO_2 levels on primary production, yield, protein, iron and zinc contents.

Parameters	Mean sum of squares			CV (%)
	Genotype (G)	CO_2 levels (T)	T × G	
Plant height (cm)	14,063**	2523 **	915 **	7.84
Days to 50 % flowering	338.7 **	69.6 **	63.4 **	2.39
Panicle length (cm)	143.39**	17.38 **	3.50	9.56
Panicle diameter (mm)	300.39 **	8.34.	6.62**	6.77
Grain yield (g)	2455.8 **	2556.8**	363.9 *	24.56
Dry fodder weight (g)	8588 **	9243 **	1131 *	21.49
Chlorophyll (μg cm^{-2})	177.7**	941.9 **	19.7 *	8.20
Flavonoids (μg cm^{-2})	0.03753 **	0.08687**	0.03753 **	8.45
Nitrogen balance index (NBI)	764 **	6211**	289 **	11.77
Grain Fe (ppm)	5834**	814**	347**	14.50
Grain Zn (ppm)	459.8**	229.00**	37.50*	12.73
Grains Protein (%)	27.60**	38.35**	2.87	18.83
Fodder Protein (%)	6.25**	7.23*	0.88	23.04

** significant at p = 0.01; * significant at p = 0.05; . Significant at p = 0.1; significant at p = .
CV - coefficient of variation

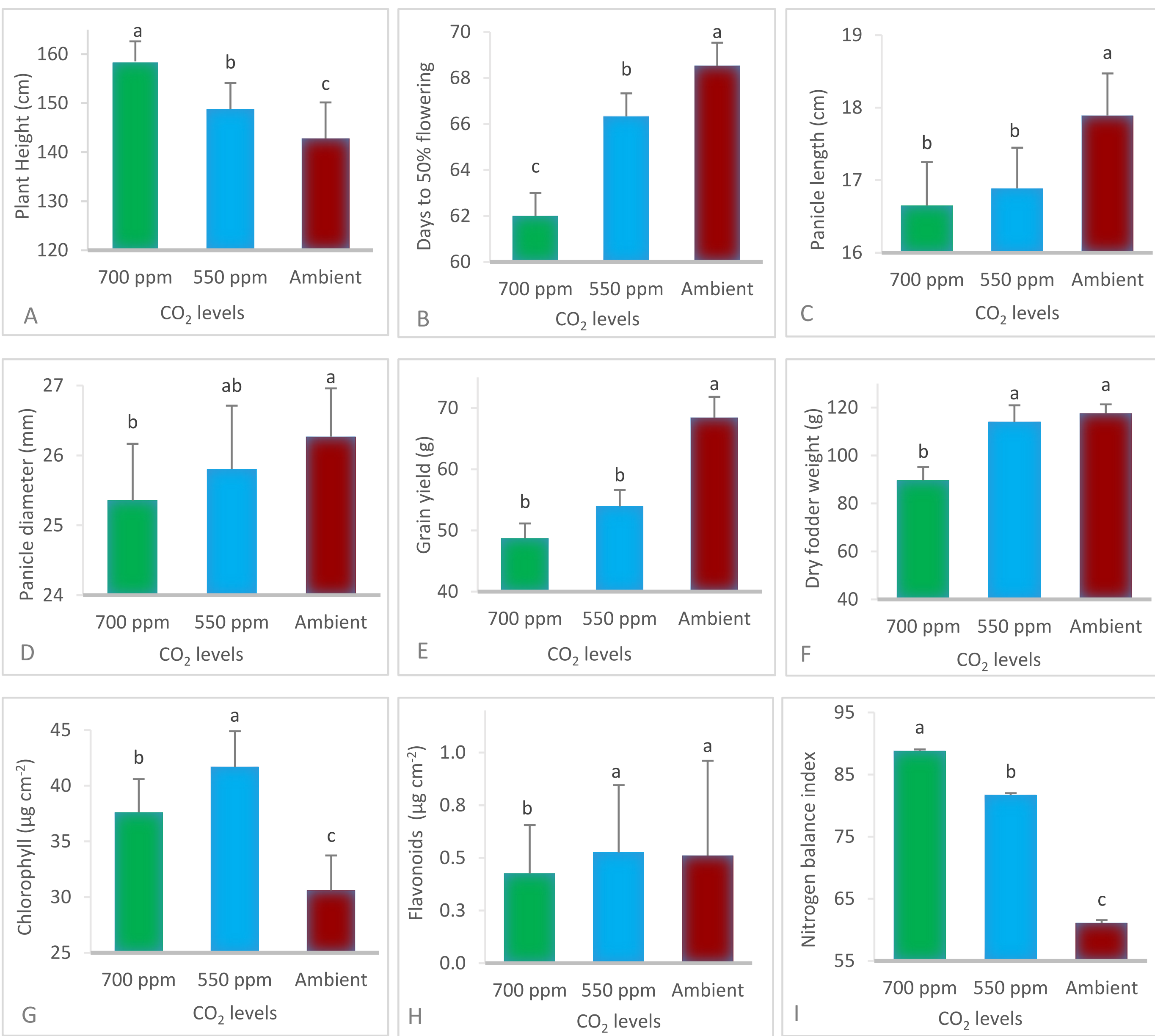

Fig. 2. Response of elevated CO_2 levels on the growth and primary production parameters of pearl millet genotypes.

production (Table 2). Average plant height was highest at 700 ppm (158.52 cm), followed by 550 ppm (148.75 cm), and ambient (142.79 cm) (Fig. 2A), showing an average increase of +8.90 % at 700 ppm and +3.61 % at 550 ppm (Table S1a). A variable response to elevated CO_2 was observed between the hybrids and inbred lines. At 700 ppm, hybrid NHB 4903 reached a height of 223.33 cm, demonstrating the highest response, whereas inbred ICMB 92888 only achieved 76.25 cm, making it the least responsive.

50 % flowering time- Interaction between 50 % flowering time and genotypic effect was significant ($P < 0.01$) (Table 2). Irrespective of genotypes, under elevated CO_2, average 50 % flowering time was reduced (Table S1a) from average 68 days–62 days with an earliest flowering at 700 ppm, followed by 550 ppm and ambient clearly indicating that increased CO_2 shortened the flowering time across all genotypes (Fig. 2B). In comparison to ambient, this led to a −9.40 % and −3.23 % decrease in 50 % flowering time at 550 and 700 ppm CO_2 levels. Most pronounced effect was noticed in HHB 67 Imp, with 48 days to reach 50 % flowering under 700 ppm followed by 53 days at 550 ppm and 62.67 at ambient, thus advancing the flowering time by 5–12 days.

Panicle length and diameter - Increasing CO_2 significantly persuaded panicle length across pearl millet genotypes ($P < 0.01$), although the interactions between genotypes and CO_2 were not significant (Table 2). The longest average panicle length was observed under ambient conditions (17.89 cm), followed by 550 ppm (16.89 cm) and 700 ppm (16.65 cm) (Fig. 2C). Notably, hybrids exhibited longer panicle lengths than the inbred lines under all conditions. The maximum panicle length was recorded in the hybrid NBH 4903 (27.25 cm) under ambient, while the shortest panicle length was observed in the inbred line ICMB 98222 (12.17 cm) at 700 ppm. On average, panicle length decreased by −7.03 % at 700 ppm and −5.09 % at 550 ppm compared to ambient conditions, indicating a more pronounced reduction at higher CO_2 (Table S1a). Further, panicle diameter also displayed significant differences among genotypes and CO_2 levels ($P < 0.1$). The highest average panicle diameter was recorded under ambient (26.27 mm), which was greater than those observed under elevated CO_2 conditions ((Table S1b and Fig. 2D).

Grain yields - Elevated CO_2 significantly reduced grain yields ($P < 0.01$) in all the pearl millet genotypes (Table 2). Average grain yield was reduced by −28.8 % (48.70 g) at 700 ppm followed by −21.2 % (53.93 g) at 550 ppm compared to ambient (68.4 g) (Table S1b and Fig. 2E). The most pronounced reductions were observed in inbred lines, with ICMR 12555 showing the highest yield loss (−51 %), followed by ICMB 92111 (−42 %), ICMB 1505 (−40 %), and ICMB 92888 (−39 %) (Fig. 3). At 550 ppm CO_2, a slight increase in grain yield was observed only in ICMR 98222, while all other genotypes experienced a decline. Conversely, hybrids ICMB 98222, 86N86, and ICMH 1301 exhibited less than −20 % reduction in grain yield at both elevated CO_2 levels.

Dry fodder weight – Dry fodder weight showed significant differences

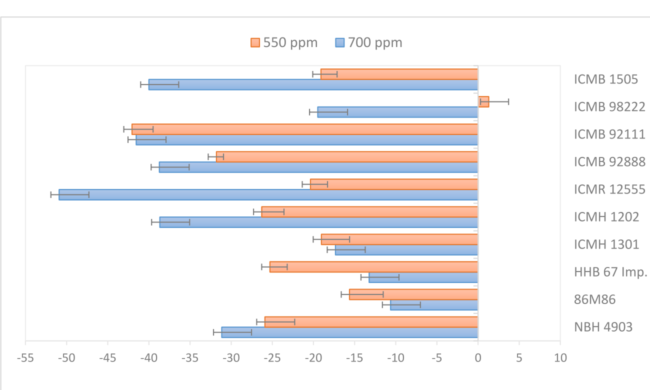

Fig. 3. Average reduction in grain yields of the pearl millet genotypes at elevated CO_2 levels to ambient conditions.

($P < 0.01$) across CO_2 and genotypes (Table 2). The highest average dry fodder weight was recorded under ambient conditions (117.53 g) followed by 114.03 g at 550 ppm and 89.63 g at 700 ppm (Fig. 2F). The reduction in dry fodder weight was more at 700 ppm (−23.74 %) compared to 550 ppm (−2.98 %) over ambient (Table S1b). Maximum dry fodder weight was recorded at 550 ppm in NBH 4903 (175.63 g) and ICMB 98222 (160.38 g) respectively. All the above results highlighted that elevated CO_2 concentrations negatively impact pearl millet's primary production parameters and grain yield, with significant reductions observed at both 550 and 700 ppm. Hybrids demonstrated greater resilience to these adverse effects as compared to inbred lines maintaining relatively higher grain yields and biomass under elevated CO_2 conditions.

3.2. *Effect of elevated CO_2 on chlorophyll, flavonoid content and nitrogen balance index*

The mean performance of the genotypes under different elevated CO_2 levels significantly ($P < 0.01$) impacted the leaf chlorophyll content, flavonoid concentration, and NBI in pearl millet genotypes (Table 2). Chlorophyll content increased substantially under elevated CO_2, with an average increase of +40.53 % (41.67 μg cm^{-2}) at 550 ppm and +29.75 % (37.60 μg cm^{-2}) at 700 ppm compared to ambient (30.59 μg cm^{-2}) (Fig. 2G). The HHB 67 Imp. Showed the highest chlorophyll content at 550 ppm (47.40 μg cm^{-2}), which decreased slightly at 700 ppm (42.77 μg cm^{-2}) and dropped significantly under ambient (35.93 μg cm^{-2}) (Table S2). Further, highest average flavonoid content was recorded at 550 ppm (0.53 μg cm^{-2}) followed by ambient conditions (0.51 μg cm^{-2}), while the lowest was observed at 700 ppm (0.43 μg cm^{-2}) (Fig. 2H). On average, flavonoid content decreased by −14.54 % at 700 ppm and increased by +5.10 % at 550 ppm compared to ambient, indicating a sensitivity to increasing CO_2. Genotype NHB 4903 demonstrated a unique response, showing an increase in flavonoid content from 0.41 μg cm^{-2} at 700 ppm to 0.42 μg cm^{-2} at 550 ppm and 0.73 μg cm^{-2} under ambient conditions. In contrast, other genotypes exhibited higher flavonoid content at 550 ppm compared to both ambient and 700 ppm. The nitrogen balance index (NBI), which reflects the relationship between chlorophyll and flavonoid, also varied significantly with CO_2 levels. The highest average NBI was observed at 700 ppm (88.82) and the lowest at ambient (61.11) conditions (Fig. 2I) (Table S5). NBI increased by 49.90 % at 700 ppm and by 38.07 % at 550 ppm compared to ambient conditions, emphasizing the role of CO_2 in maintaining physiological balance in pearl millet (Table S2).

3.3. *Effect of elevated CO_2 on grains iron and zinc content*

A large variability was observed in both grain Fe and Zn content across genotypes and CO_2 levels, with Fe content ranging from 38.80 to 121.38 ppm and Zn content from 24.40 to 51.38 ppm (Table S3). Grain Fe and Zn contents were significantly ($P < 0.01$) influenced by genotypes, CO_2 and their interactions, except for the interactions of grain Zn with genotypes and CO_2 levels, which was significant at $P < 0.05$ (Table 2). Data indicated that average Fe and Zn content in grains increased till 550 ppm and a declining trend was noticed at 700 ppm.

Grain Fe content – The average grain Fe content was highest at 550 ppm (75.55 ppm), showing a +4.25 % increase over ambient conditions, while the lowest was recorded at 700 ppm CO_2 (67.11 ppm), reflecting a −4.01 % decrease compared to ambient conditions (Fig. 4A) (Table S3). Among the genotypes, the high-Fe inbred line ICMB 1505 remained stable and had the highest grain Fe content (121.41 ppm) at 550 ppm, at par with ambient (121.38 ppm) and slightly reduced at 700 ppm CO_2 (110.51 ppm). Interestingly, low-Fe genotypes such as ICMB 92111, ICMB 92888, HHB 67 Imp., and NBH 4903 exhibited increased Fe content under elevated CO_2 conditions. At 550 ppm, the highest percent increase in Fe content over ambient was observed in 86M86 (23.11 %), NBH 4903 (21.88 %), and ICMB 92111 (+16.78 %), while a drastic

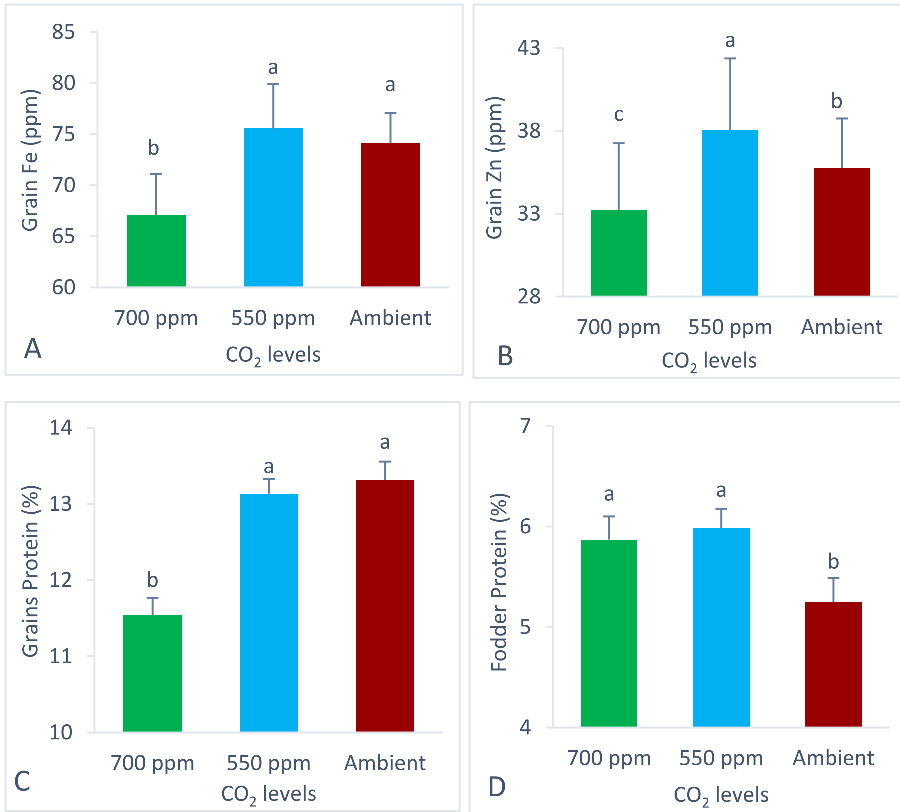

Fig. 4. Effect of elevated CO_2 levels on the protein, iron, zinc contents in the pearl millet genotypes.

reduction was noted in ICMH 1301 (16.48 %) (Fig. 5A). Conversely, at 700 ppm, Fe content decreased in most genotypes compared to ambient conditions, with the highest reductions recorded in ICMB 98222 (40.61 %), ICMH 1301 (23.88 %), and ICMH 1202 (13.33 %). However, genotypes such as ICMB 92888 (31.94 %) and ICMB 92111 (17.63 %) showed increased Fe content at 700 ppm.

Gain Zn content – The impact of elevated CO_2 on Zn concentration varied across genotypes, showing both increases and decreases relative to ambient conditions. Like grain Fe, Zn content increased across all genotypes up to 550 ppm but declined at 700 ppm (Fig. 4B). Notably, 86M86, ICMR 12555, ICMB 98222, ICMB 92111, and ICMB 1505 exhibited higher Zn concentrations at 550 ppm. However, significant reductions were observed at 700 ppm in hybrids ICMH 1301, ICMB 98222, and ICMH 1202 (Fig. 5B). Among inbred lines, ICMB 1505 recorded the highest Zn content at both 550 ppm (51.38 ppm) and 700 ppm (49.12 ppm) followed by ICMB 98222 (46.23 ppm) and ICMR 12555 (44.74 ppm) (Table S3).

3.4. *Effect of elevated CO_2 on grain and fodder protein content*

There were highly significant differences in both grain and fodder protein content among genotypes ($P < 0.01$) and across CO_2 levels for grain ($P < 0.01$) and fodder ($P < 0.05$) proteins, while the interaction effects between genotypes and CO_2 were not significant (Table 2). Grain protein content across genotypes under different CO_2 levels ranged from 9.67 % to 16.19 % (Table S3). Elevated CO_2 resulted in a reduction in grain protein content, with an average decrease of −11.54 % at 700 ppm compared to ambient conditions, amounting to an overall reduction of −13.40 % (Fig. 4C). Grain protein accumulation in inbred lines was relatively unaffected by higher CO_2, while it decreased significantly in hybrids. The maximum grain protein content was observed in ICMB 1505 (16.19 %) under ambient, but it dropped to 12.78 % at 700 ppm. The lowest grain protein content at 700 ppm was recorded in ICMR 12555 (8.67 %), followed by ICMH 1301 (9.52 %) and 86M86 (9.57 %). Interestingly, HHB 67 Imp. was the only genotype in which grain protein content increased under elevated CO_2, reaching 14.34 % at 550 ppm and 14.22 % at 700 ppm compared to 13.92 % under ambient (Table S3). Further, percent reduction in grain protein content over ambient was more at 700 ppm compared to 550 ppm (Fig. 6A). Genotypes such as ICMH 1301, ICMR 12555, ICMH 1202, and ICMB 1505 showed drastic reductions in grain protein content. Conversely, only in HHB 67 Imp. and ICMB 92888 exhibited increases in grain protein under 700 ppm. On the other hand, grain protein content improved at 550 ppm, in a few genotypes including 86M86, HHB 67 Imp., ICMB 98222, ICMB 92111, ICMB 92888, ICMR 12555 and in few genotypes decreased slightly.

The average fodder protein content increased slightly but was statistically at par with elevated CO_2 compared to ambient, with increase of +5.99 % at 550 ppm and +5.87 % at 700 ppm (Fig. 4D). The maximum fodder protein content was recorded in ICMB 92111 (7.81 %) at 550 ppm, while the lowest was observed in ICMB 92888 (4.00 %) under ambient (Table S3). The percent changes in fodder protein accumulation were maximum at 700 ppm followed by 550 ppm over ambient (Fig. 6B). Among the genotypes, fodder protein content increased at 700 ppm in HHB 67 Imp., ICMB 92888, 86M86, and ICMH 1301, whereas ICMB 92111 and NBH 4903 exhibited decreases. At 550 ppm, fodder protein content increased in all genotypes except ICMB 1505. Most hybrids demonstrated superior performance in terms of fodder protein accumulation compared to the parental lines. These results suggest that elevated CO_2 reduces grain protein content while enhancing fodder protein content in most genotypes, though responses vary depending on CO_2 concentration and genotype.

3.5. *Correlation and genotypic attribution for CO_2 resilient traits*

Phenotypic correlation among primary production parameters and micronutrient traits in pearl millet revealed both positive and negative

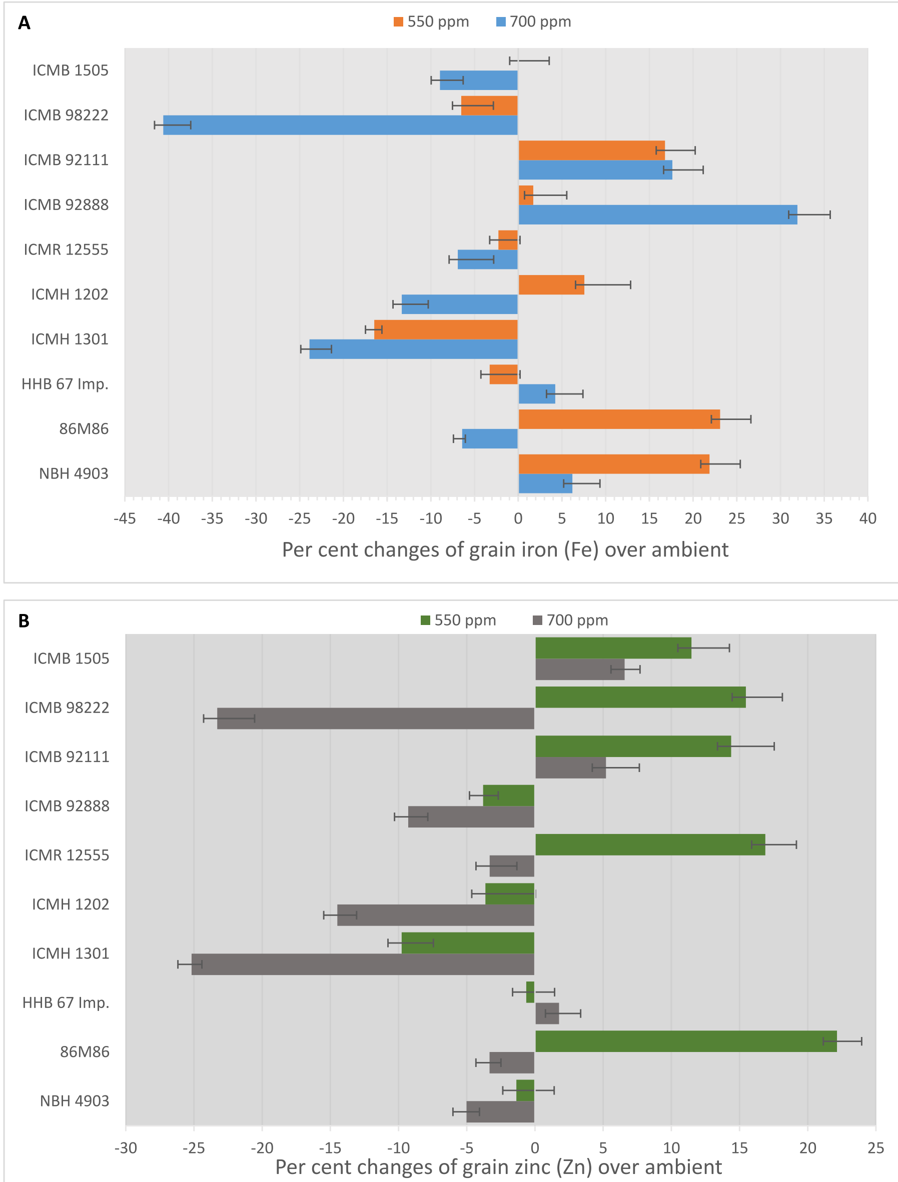

Fig. 5. Average reduction in iron and zinc contents of the pearl millet genotypes at elevated CO_2 levels to ambient conditions.

correlations under elevated CO_2 levels at 550 ppm and 700 ppm (Table S4). At 550 ppm, plant height correlated positively with most traits, except for grain and fodder protein, when compared to ambient conditions. Highly significant positive correlations were observed between panicle diameter, grain yield, and dry fodder weight ($r = 0.82$ and 0.86, $P < 0.01$). Additionally, Fe and Zn content were strongly correlated with grain yield, with a significant association between Fe and Zn content ($r = 0.84$, $P < 0.01$). Grain and fodder protein content were positively correlated with Fe and Zn, as well as with each other ($r = 0.45$). At 700 ppm, panicle length showed significant positive correlations with both grain yield and dry fodder weight ($r = 0.72$ and 0.70, $P < 0.05$), while grain yield and dry fodder weight remained strongly correlated ($r = 0.81$, $P < 0.01$). The positive association between Fe and Zn content persisted ($r = 0.81$, $P < 0.01$). Grain protein correlated positively with flavonoid content, Fe, and Zn, while fodder protein showed positive correlations with Fe and Zn at 700 ppm CO_2.

The top-performing genotypes for each attribute under different CO_2 were identified using multiplicative interactions analysis (Table 3). Among them, high Fe hybrid ICMH 1301 emerged as the most productive under both ambient and elevated CO_2 conditions (700 ppm), making it a robust candidate for high-yield resilience. Similarly, 86M86, ICMB 98222, and ICMH 1202 exhibited strong adaptability and maintained high grain yield under tested CO_2 levels. For grain nutritional quality, ICMB 1505 consistently exhibited superior Fe and Zn accumulation, highlighting its resilience in micronutrient retention at elevated CO_2. Additionally, ICMB 1505 and ICMB 92111 were the most stable in grain protein content, while HHB 67 Imp., ICMB 1505, ICMB 92888, and ICMB 92111 ranked highest in fodder protein accumulation. These findings underscore the potential of specific genotypes candidature for yield and nutrition genetic enhancement program in sustaining productivity and nutritional quality under changing CO_2 conditions, making them valuable for future breeding programs targeting climate resilience.

4. Discussion

Plant biochemistry is significantly impacted by rising atmospheric CO_2 levels, which has important ramifications for food quality and nutrition. Staple crops like wheat, rice, barley, oats, potatoes, and other tree species frequently lose nutritional value when CO_2 levels [11,13,16,

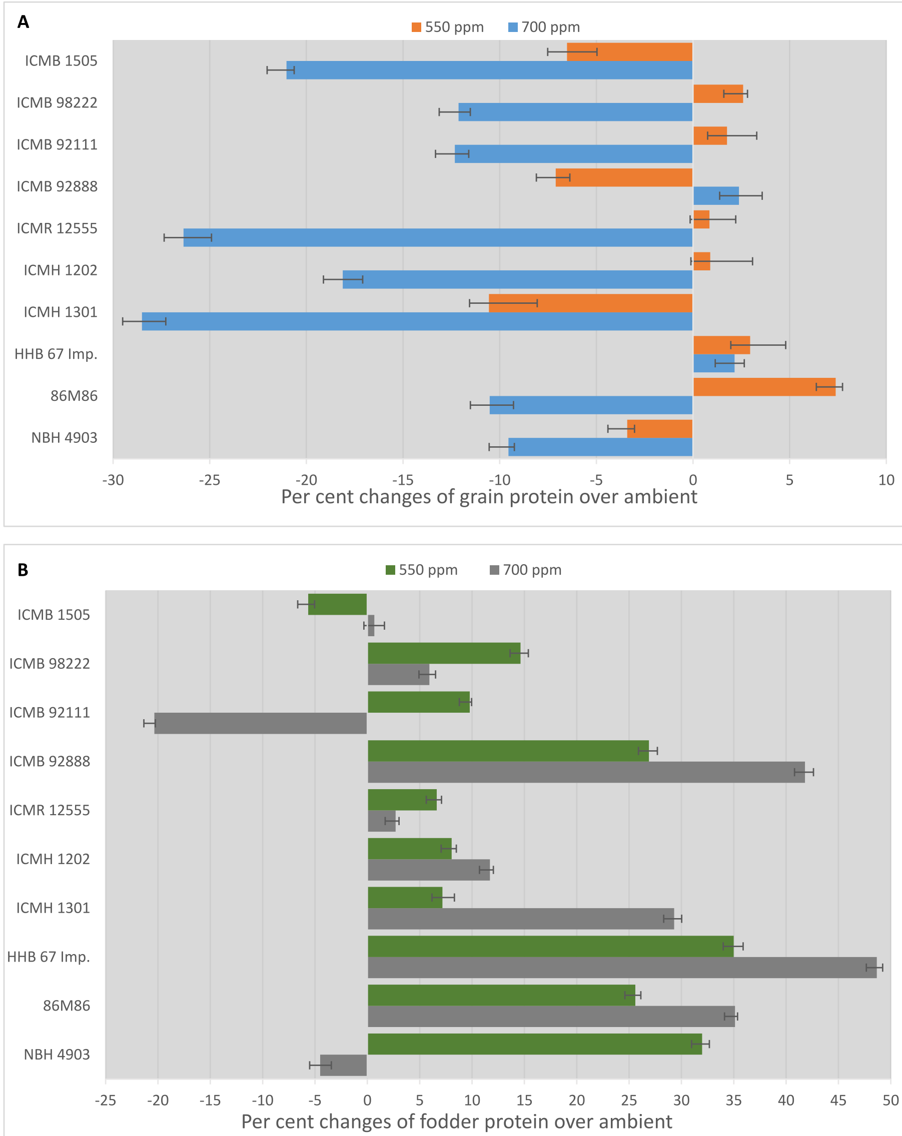

Fig. 6. Per cent reduction of protein content in the pearl millet genotypes at elevated CO_2 levels to ambient conditions.

18]. Within the same species, cultivars may differ in nutrient concentrations due to both small-scale physiological characteristics and large-scale variations in C3 and C4 photosynthetic pathways [41]. Along with maize and sorghum, pearl millet contributes up to 30 % of terrestrial carbon fixation and is a highly climate-resilient C4 crop [42,43]. Improving output and nutritional quality of pearl millet requires a better understanding of how the crop reacts to increased CO_2. This study demonstrates how CO_2 significantly affects the primary production and grain quality of pearl millet in hybrids and parental lines.

Elevated CO_2 levels significantly influenced plant height and maturity in pearl millet, leading to increased height and earlier flowering in most genotypes, except for ICMB 92888 and ICMR 12555 at 550 ppm and 700 ppm CO_2. These changes are attributed to enhanced photosynthetic activity under higher CO_2. Previous studies have reported variable effects on plant height, including reductions [44], increases [45,46], and no significant changes [47]. These varying outcomes to elevated CO_2 levels were attributed to the complex and species-specific nature of plant responses. In this study, yield-related traits such as panicle length, panicle diameter, grain yield, and dry fodder weight were negatively impacted by elevated CO_2; hybrids exhibited greater resilience than parental lines, maintaining relatively higher yields and biomass. Climate change is expected to further influence grain yield and quality in the future [48]. Plant responses to environmental factors, including CO_2 and temperature, are largely governed by carbon source-sink balance [49], and an imbalance may constrain CO_2 fertilization effects on growth and yield [50].

Carbon source-sink balance theory for pearl millet under elevated CO_2 conditions suggests that as CO_2 levels increase, the plant's carbon assimilation (source) might be greater than its carbon allocation to sinks (sowing and other plant parts). This could lead to changes in how carbon is distributed within the plant, potentially impacting grain yield and nutritional compositions [51]. Elevated CO_2 increases photosynthesis and biomass but potentially impacts grain yield and grain nutrition composition, while high temperatures can negatively affect photosynthesis and sink strength. Overall, the source-sink balance in pearl millet is a complex interplay of environmental factors and plant physiology, impacting the ability of sink tissues to utilize sugars, leading to a mismatch between source (photo-assimilates) and sink (sink tissue) [52]. Additionally, the impact of elevated CO_2 on soil organic carbon storage depends on the balance between changes in inputs and turnover. Root-microbe-mineral interactions in the rhizosphere play a crucial role in regulating plant growth and development under changing CO_2

Table 3
Ranking of top three entries for climate resilient traits.

Parameters	Rank	Simulated CO_2 levels (ppm)		
		700	550	415
Plant height	1	NBH 4903	86M86	86M86
	2	HHB 67 Imp.	ICMH 1301	HHB 67 Imp.
	3	86M86	HHB 67 Imp.	NBH 4903
days to 50 % flowering	1	HHB 67 Imp.	HHB 67 Imp.	ICMB 1505
	2	ICMH 1202	ICMB 1505	ICMR 12555
	3	ICMB 1505	ICMR 12555	HHB 67 Imp.
Panicle length	1	NBH 4903	NBH 4903	NBH 4903
	2	HHB 67 Imp.	86M86	HHB 67 Imp.
	3	ICMH 1202	ICMH 1301	86M86
Panicle diameter	1	ICMH 1301	ICMB 98222	ICMH 1301
	2	ICMB 98222	ICMH 1301	ICMB 98222
	3	NBH 4903	86M86	NBH 4903
Grain yield	1	ICMH 1301	ICMB 98222	ICMH 1301
	2	86M86	ICMH 1301	ICMH 1202
	3	ICMB 98222	86M86	86M86
Dry fodder weight	1	86M86	NBH 4903	ICMH 1301
	2	ICMH 1301	ICMB 98222	NBH 4903
	3	NBH 4903	86M86	86M86
Chlorophyll	1	HHB 67 Imp.	HHB 67 Imp.	ICMH 1301
	2	NBH 4903	ICMH 1301	NBH 4903
	3	ICMH 1301	NBH 4903	HHB 67 Imp.
Flavonoids	1	HHB 67 Imp.	ICMH 1301	NBH 4903
	2	86M86	HHB 67 Imp.	HHB 67 Imp.
	3	ICMH 1301	ICMB 92888	ICMH 1301
Nitrogen balance index	1	NBH 4903	NBH 4903	ICMH 1202
	2	ICMH 1202	ICMB 98222	ICMB 98222
	3	ICMH 1301	ICMH 1202	86M86
Grain Fe	1	ICMB 1505	ICMB 1505	ICMB 1505
	2	ICMR 12555	ICMB 98222	ICMB 98222
	3	ICMH 1301	ICMH 1202	ICMH 1301
Grain Zn	1	ICMB 1505	ICMB 1505	ICMB 1505
	2	HHB 67 Imp.	ICMB 98222	ICMB 98222
	3	ICMR 12555	ICMR 12555	ICMR 12555
Grains Protein	1	ICMB 1505	ICMB 92111	ICMB 92111
	2	HHB 67 Imp.	ICMR 12555	ICMB 1505
	3	ICMR 12555	ICMB 98222	ICMR 12555
Fodder Protein	1	HHB 67 Imp.	ICMB 1505	ICMB 1505
	2	ICMB 92888	ICMB 92111	ICMB 92111
	3	ICMB 92111	ICMB 98222	ICMB 98222

conditions [53–55].

Under elevated CO_2, chlorophyl—a key pigment in photosynthesis—showed a substantial increase, with hybrids exhibiting higher levels than parental lines. Among the tested genotypes, HHB 67 Imp. recorded the highest chlorophyll content at 550 ppm CO_2, reinforcing previous findings that C4 plants, including pearl millet, benefit from CO_2 enrichment [56]. The positive effects of elevated CO_2 on photosynthesis are influenced by environmental factors such as temperature [57], soil water availability [58], and vapor pressure deficit [59]. For instance, photosynthesis was increased in maize under CO_2 enrichment at 25/19 °C to 31/25 °C, but declined significantly at 37/31 °C, demonstrating temperature as a limiting factor [60]. Additionally, the efficiency of Rubisco, a key enzyme in C4 photosynthesis, is highly temperature-dependent [61]. While elevated CO_2 can enhance photosynthetic activity, it may also reduce plant nutrient demand relative to carbon accumulation, leading to reduced root uptake and dilution of essential nutrients such as nitrogen, iron, and zinc [12].

Flavonoids are vital for plant growth, development, and defence against biotic and abiotic stresses while also enhancing nutritional quality [62]. In pearl millet, flavonoid content peaked at 550 ppm CO_2 but declined at 700 ppm. In contrast, chickpea has shown no significant changes in flavonoid content under elevated CO_2 [36]. The NBI, which reflects the relationship between chlorophyll and flavonoid levels, increased under elevated CO_2. NBI exhibited positive correlation with chlorophyll and a negative correlation with flavonoid content, suggesting that elevated CO_2 may enhance nitrogen utilization efficiency or nitrogen fixation in plants [63].

At elevated CO_2, Fe content increased in some of the low-Fe genotypes, including parental lines (ICMB 92111 and ICMB 92888) and hybrids (HHB 67 Imp. and NBH 4903), while it declined in some of the high-Fe genotypes such as parental lines (ICMB 1505 and ICMB 98222) and hybrids (ICMH 1202 and ICMH 1301). This proves the positive and negative effects are largely genotypic dependent. This aligns with previous studies, which reported significant reductions in Fe and Zn concentrations in wheat, rice, peas, and soybeans, based on a meta-analysis of 143 crops grown under elevated CO_2 conditions [16, 23]. This phenomenon is frequently explained by the dilution effect, which states that greater CO_2 causes plants to grow more, which raises the demand for nutrients and may lower the content of those nutrients in grains [13]. Previous studies reported a dilution effect between yield and Fe/Zn in pearl millet [27,28,38,64]. Rising CO_2 are expected to further decrease the availability of protein, iron, and zinc, potentially from pearl millet, worsening nutrient deficiencies across Asia and Africa [65]. For example, study conducted by the International Food Policy Research Institute (IFPRI) predicted that by 2050, the rise in CO_2 will lessen the protein availability by 20 %, while iron and zinc would be reduced by 15 % in different crops [65].

Under elevated CO_2, fodder protein content increased, whereas grain protein content declined. Similar reductions in protein concentration, ranging from 9.8 % to 15.3 %, have been reported in barley, potato, rice, and wheat under elevated CO_2 conditions [11,41,66]. Among the tested genotypes, HHB 67 Imp. and ICMB 92888 maintained stable grain protein levels across different CO_2 levels while also exhibiting higher chlorophyll and flavonoid content. The reduction in grain protein content under CO_2 is commonly attributed to the dilution effect, where increased carbohydrate accumulation leads to a decrease in protein concentration [48,67]. Similar trends have been observed in soybean grown under elevated CO_2, where grain protein content declined [23]. It is well established that in small-grain cereals like rice, wheat, and barley, up to 90 % of nitrogen is mobilized from vegetative tissues to grains during grain filling [68]. Consequently, reduced nitrogen investment in plants under CO_2 may be a key factor contributing to lower grain protein concentrations.

In conclusion, higher CO_2 boosted plant height, leaf chlorophyll content, and plant maturity. However, grain production and biomass were drastically decreased, with hybrids demonstrating greater resilience and retention of current yields compared to parental lines, which were more severely impacted. Fe and Zn content in grain showed a slight increase at 550 ppm but decrease at 700 ppm. Entries HHB 67 Imp., NBH 4903, ICMB 92111, and ICMB 92888 exhibited increased Fe and Zn accumulation, whereas ICMH 1301, ICMH 1202, and ICMB 98222 experienced reductions at extreme CO_2 (700 ppm) expected in 2050. Under elevated CO_2, grain protein content decreased across all genotypes, while fodder protein content increased. Studies suggest that accelerated biomass accumulation under elevated CO_2 may dilute nutrient concentrations, resulting in reduced grain protein, Fe and Zn levels both in C_3 and C_4 plants. This study underscores the need for regular monitoring of yield and nutritional traits such as protein, Fe, and Zn under changing climate to improve grain production without further decline in nutrition levels of a Nutri-cereal. Prospecting increased CO_2 will exacerbate hidden hunger, especially in vulnerable populations or slow progress in addressing iron and zinc deficiencies in populations that rely on pearl millet as a staple food. Biofortified varieties may offer partial resilience, but their efficacy under future climate scenarios requires further study.

Based on the observed differential responses to changing CO_2 levels in this study, there is a need to prioritize and mainstream nutritional traits in target product profiles (TPPs) through appropriate breeding techniques and comprehensive germplasm evaluation to develop varieties that are well adapted to future climate scenarios. Using high-performing lines like ICMB 98222, ICMB 1505, and ICMR 12555, as well as parents of ICMH 1301 and HHB67, can lead to climate-resilient products with improved yield and nutrition under rising CO_2 levels.

Therefore, further investigation of a wider set of varieties to screen for potential tolerance to increased CO_2 levels and maintain nutritional benefit of this crop is recommended.

CRediT authorship contribution statement

Mahalingam Govindaraj: Writing – review & editing, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **Ramanagouda Gaviyappanavar:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Avijit Tarafdar:** Writing – original draft, Investigation, Data curation. **Raju Ghosh:** Methodology, Investigation. **Sean Mayes:** Writing – review & editing, Resources. **Pooja Bhatnagar-Mathur:** Writing – review & editing, Conceptualization. **Mamta Sharma:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2025.102124.

Data availability

Data will be made available on request.

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