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Individual and Interactive Effects of Multiple Abiotic Stress Treatments on Early-Season Growth and Development of Two *Brassica* Species

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Abstract: Potential global climate change-related impacts on crop production have emerged as a major research priority and societal concern during the past decade. Future changes, natural and human-induced, projected in the climate have implications for regional and global crop production. The simultaneous occurrence of several abiotic stresses instead of stress conditions is most detrimental to crops, and this has been long known by farmers and breeders. The green leafy vegetables of the Brassicaceae family have especially gained attention due to their many health benefits. However, little information is available about abiotic stress's effects on *Brassica* vegetables' growth and development. An experiment was conducted on two *Brassica* species: *B. oleracea* L. var. acephala WINTERBOR F1 (hybrid kale) and *B. juncea* var. GREEN WAVE OG (mustard greens). Seven treatments were imposed on the two brassica species in soil–plant–atmosphere–research (SPAR) units under optimum moisture and nutrient conditions, including a control treatment (optimal temperature and UV-B conditions at ambient CO_2 levels), and six treatments where stresses were elevated: CO_2, UV-B, temperature (T), CO_2+UV-B, CO_2+T, and CO_2+UV-B+T. Above- and below-ground growth parameters were assessed at 26 d after sowing. Several shoot and root morphological and developmental traits were evaluated under all the treatments. The measured growth and development traits declined significantly under individual stresses and under the interaction of these stresses in both the species, except under elevated CO_2 treatment. All the traits showed maximum reductions under high IV-B levels in both species. Leaf area showed 78% and 72% reductions, and stem dry weight decreased by 73% and 81% in kale and mustard, respectively, under high UV-B levels. The increased CO_2 concentrations alleviated some deleterious impacts of high temperature and UV-B stresses. The results of our current study will improve our understanding of the adverse effects of environmental stresses on the early-season growth and development of two *Brassica* species.

Keywords: temperature stress; elevated CO_2; UV-B; *Brassica oleracea*; *Brassica juncea*

1. Introduction

Many scientific and intergovernmental reports warn of the dangerous consequences of climate change for various aspects of human life, with considerable threats to plant productivity [1–7]. Current temperatures are approximately 1 °C above pre-industrial levels, and a further increase in global temperatures by 0.5 °C would elevate the related risks [6]. Elevated atmospheric CO_2 is the most eminent cause of global warming. At present, the global atmospheric CO_2 concentration is 417 ppm (recorded in March 2021 by Mauna Loa observatory, Waimea, HI, USA), which was only 270 ppm during the pre-industrial era. Over the last two centuries, such an unprecedented rise in atmospheric CO_2 has occurred due to massive anthropogenic activities such as deforestation, fossil-based fuel combustion, rapid urbanization, and industrialization [8]. Climate change is a cumulative effect of multiple factors, such as changes in temperatures, radiation, precipitation, and CO_2 levels [9]. Thus, it is imperative to understand the combined effects of multiple factors and elevated CO_2 to mimic real-world situations.

There has been an increasing trend in consuming more green leafy vegetables in the human diet. Among the variety of green leafy vegetables available for human consumption, kale (*Brassica oleracea* L.) and mustard (*Brassica juncea* L.) are among the most consumed vegetables [10]. The vegetables of the *Brassicaceae* family gained attention due to their sulfur-containing phytonutrients, known as glucosinolates, that are known to promote health. Glucosinolates, flavonoids, and phenolic compounds are responsible for antioxidant and free radical scavenging properties [11,12]. Kale leaves are generally consumed fresh and unprocessed as a salad or cooked and used as a garnish, and they are usually sold in fresh, canned, and frozen forms [13]. Kale is reported to have much higher protein than other *Brassica* family vegetables [14] and other green leafy vegetables such as spinach (2.9% on a fresh weight basis). Both kale and mustard are excellent sources of vitamin A and β-carotenes, and flavonoids [15]. Research studies have reported other health-beneficial activities of kale and a mustard-like protective role in coronary artery diseases, anti-inflammatory activities, antigenotoxic ability, and gastroprotective activity [10]. The presence of compounds such as polyphenols, glucosinolates, carotenoids, and vitamins E and C in kale and mustard is associated with cardiovascular protection [16], and mustard is also beneficial in the treatment of diabetes and cataracts [17].

Both kale and mustard are considered cool-season crops, generally thriving at daytime temperatures of 18 to 24 °C and nighttime temperatures of 4 to 7 °C [18]. Kale and mustard are sensitive to high temperatures [19]. Thus, an early-season planting can help prevent high temperatures during the seedling stage and mitigate the losses due to stress. Since abiotic stresses are interconnected, their concurrent occurrence and combined effects have been shown to be more destructive to plant growth, productivity, and yield, and will be essential in devising management and breeding decisions in the coming years.

Several previous studies on elevated CO_2 concentration in crops have revealed a significant direct impact on plant growth and crop yield that can compensate for a potentially hotter climate. Even though it has been noticed that an increase in CO_2 concentration leads to a significant yield increase in C_3 plants [20–22], few direct effects have been recorded on kale and mustard plants. However, the impact of elevated CO_2 on these two *Brassica* species, much less the interaction of elevated CO_2, temperature, and UV-B, is not adequately understood to allow accurate predictions of future crop production.

The projected higher doses of incoming UV-B radiation have been reported to stimulate various responses of higher plants [23,24]. Some of the deleterious effects of UV-B radiation on plants include DNA damage, the disintegration of cellular membranes, photooxidation of leaf pigments and phytohormones, and inhibition of photosynthesis [25–27]. Moreover, higher UV-B radiation can affect whole-plant photosynthesis via alterations in leaf thickness, anatomy, and canopy morphology [28]. Therefore, to optimize the production with suitable management and breeding strategies for green leafy vegetables in the future, it is crucial to understand the effects of UV-B radiation and the other stresses on these crops. Although little data exists on the growth, development, and productivity of crops in response to CO_2, temperature, or UV-B applied alone, to the best of our knowledge no data is available on the interactive effects of multiple factors on the growth and development of kale and mustard.

Root systems are challenging to study because of their highly structured underground distribution, the complexity of vigorous interactions with the environment, and their diversity of functions. The root system can be more affected than the aerial parts by multiple abiotic stresses. Despite this, the influence of abiotic stresses on root development has been considerably less studied than on shoots because of the limited accessibility of root observations [29]. Different methodologies have been developed to study root growth under both field and controlled environmental conditions. Root scanning based on the WinRHIZO optical scanner [30] is an efficient method that allows image analysis and examination of the root morphological traits. This technique provides data that, using established software protocols, enables quick analysis and rapid, straightforward, and accurate screening of root characteristics. Therefore, this method is most suited for screening the root traits of kale and mustard plants grown under controlled environmental conditions.

Previous studies on sunlit, controlled environment chambers demonstrated the effects of multiple abiotic stress interactions on plant growth, development, physiology, and reproduction in cotton, *Gossypium hirsutum* L. [31], soybean, *Glycine max* (L.) Merr. [32,33], and some other crops. Still, none of these studies have been conducted on kale and mustard. One of the studies performed on soybean revealed that plant height and leaf area development were the most sensitive processes in responses to multiple stresses, leading to a profound loss in biomass production [33]. However, it was observed that elevated CO_2 concentration ameliorated the damaging effects caused by various abiotic stresses on most of the plant growth and physiological parameters. According to Reddy et al. [34], numerous environmental stresses affect crop growth, development, and physiological processes multiplicatively, not additively. Hence, rather than a particular stress condition, the simultaneous occurrence of multiple abiotic stresses is most harmful to any crop. Therefore, the interactive effects of various environmental stresses on kale and mustard must be sufficiently understood to allow accurate predictions of future crop production. The objectives of this study were to characterize the changes in vegetative growth and developmental traits in kale and mustard in their response to multiple environmental factors of (CO_2) [400 and 720 μmol mol^{-1} (+(CO_2)], temperature treatments [25/17 °C and 35/27 °C (day/night) (+T)], and B radiation [0 and 10 kJ m^{-2} d^{-1} (+UV-B)] during early-season growth.

2. Materials and Methods

2.1. Seed Material and Experiment Conditions

Brassica species: *B. oleracea* var. WINTERBOR F1 (hybrid kale) and *B. juncea* var. GREEN WAVE OG (mustard greens) were used for this study. The experiment was conducted in August 2019 in sunlit soil–plant–atmosphere–research (SPAR) chambers located at the Rodney Foil Plant Science Research facility of Mississippi State University, Mississippi State, MS (lat. 33°28′ N, long. 88°47′ W). Each SPAR chamber consists of a steel soil bin and a 1.27 cm thick Plexiglas chamber to accommodate root and aerial plant parts. The Plexiglas allows 97% of the visible solar radiation to pass without spectral variability in absorption (wavelength 400–700 nm). During the experiment, the incoming daily solar radiation measured with a pyranometer (Model 4–8; The Eppley Laboratory Inc.) outside the SPAR units ranged from 11.3 to 31.3 MJ m^2 d^{-1} with an average value of 25.10 ± 0.82 MJ m^2 d^{-1}. More details of the SPAR chamber operations and control have been described by Reddy et al. [34]. Briefly, air ducts on each SPAR unit's northern side were connected to the heating and cooling devices. Conditioned air was passed through the plant canopy with sufficient velocity to cause leaf flutter (4.7 km h^{-1}) and was returned to the air-handling unit just above the soil level. Two electrical resistance heaters provided short heat pulses as needed to fine-tune the air temperature control. Chamber air temperature, CO_2 concentration, soil watering in each SPAR unit, and continuous monitoring of environmental and plant gas exchange variables were controlled by a dedicated computer system [35] (Table 1). The vapor pressure deficits in the units were estimated from these measurements as per Murray [36] (Table 1).

Table 1. The set treatments and measured day, night, and average temperatures, chamber carbon dioxide concentration (CO_2), daytime and nighttime vapor pressure deficit (VPD), and evapotranspiration (ET) during the experimental period of each treatment in kale and mustard.

Treatments	Measured Temperature (°C)			CO_2 (µmol mol^{-1})	VPD (kPa)		Mean ET (L H_2O d^{-1})
	Day	Night	Day/Night	Day	Day	Night	Day/Night
Control	24.8 ± 0.03	17.5 ± 0.04	21.6 ± 0.03	434.3 ± 1.77	1.4 ± 0.01	0.98 ± 0.01	8.6 ± 0.45
+CO_2	25 ± 0.03	17.6 ± 0.03	21.8 ± 0.02	723.6 ± 0.33	1.4 ± 0.01	1 ± 0.01	7.4 ± 0.67
+T	30 ± 0.88	22.4 ± 0.88	26.7± 0.87	435.1 ± 1.76	2.1 ± 0.15	1.5 ± 0.11	8.5 ± 0.63
+UV-B	24.7 ±0.04	17.4 ± 0.04	21.5 ± 0.03	436.9 ± 2.44	1.4 ± 0.01	1 ± 0.01	6.6 ± 0.24
+T+CO_2	30.3 ± 0.94	22.6 ± 0.93	27 ± 0.92	724 ± 0.36	2.6± 0.15	1.7 ± 0.12	8.7 ± 0.81
+UV-B+CO_2	24.8 ± 0.03	17.4 ± 0.03	21.5 ± 0.03	720.5 ± 0.55	1.3 ± 0.02	0.93 ± 0.01	5.2 ± 0.47
+UV-B+CO_2+T	30 ± 0.89	22.4 ± 0.88	26.7 ± 0.87	733.2 ±0.49	2.6 ± 0.19	1.8 ± 0.14	6.91 ± 0.59

During the experiment, the incoming daily solar radiation measured with a pyranometer (Model 4–8; The Eppley Laboratory Inc., Newport, RI, USA) outside the SPAR units ranged from 11.3 to 30.9 MJ m^2 d^{-1} with an average value of 24.12 ± 1.14 MJ m^2 d^{-1}.

Seeds were sown in 210 polyvinyl-chloride pots (15.2 cm diameter and 30.5 cm height) filled with the soil medium consisting of 3:1 sand/topsoil classified as a sandy loam (87% sand, 2% clay, and 11% silt) with 500 g of gravel at the bottom of each pot. Initially, three seeds were sown in each pot, and 7 d after emergence the plants were thinned to one per pot. Pots were arranged in 10 rows with three pots per row in each SPAR chamber with alternating kale and mustard plants. Plants were irrigated three times a day through an automated, computer-controlled drip system with full-strength Hoagland's nutrient solution [37], delivered at 0700, 1200, and 1700 h, based on treatment-based evapotranspiration values. Evapotranspiration rates expressed on a ground area basis (L d^{-1}) throughout the treatment period were measured in each SPAR unit as the rate at which the cooling coils removed the condensate at 900 s intervals [35,38,39]. They were obtained by measuring the mass of water in collecting devices connected to a calibrated pressure transducer. Average evapotranspiration values for each treatment during the experimental period are provided in Table 1.

2.2. Treatments

The treatments included combinations of two [CO_2], [400 and 720 µmol mol^{-1} (+CO_2)], two different temperatures, [25/17 °C and 35/27 °C (+T) (day/night)], and two daily biologically effective UV-B radiation intensities, [0 and 10 kJ m^{-2} d^{-1} (+UV-B)].

The control treatment was 400 µmol mol^{-1} [CO_2], 25/17 °C (day/night) temperatures, and 0 kJ m^{-2} d^{-1} UV-B treatment. All SPAR chambers were maintained at control conditions until 12 days after sowing (DAS). Subsequently, each chamber was set at one of the seven treatments until the final harvest (26 DAS; 14 DAT): (1) a control treatment with optimum temperature, ambient CO_2 levels, and no UV-B; (2) optimum temperature with elevated CO_2 levels and no UV-B (+CO_2); (3) elevated temperature with ambient CO_2 levels and no UV-B (+T); (4) optimum temperature and ambient CO_2 levels with 10 kJ UV-B (+UV-B); (5) elevated temperature, and CO_2 levels with no UV-B (+T+CO_2); (6) optimum temperature with elevated CO_2 levels and 10 kJ UV-B (+CO_2+UV-B); (7) elevated temperature and elevated CO_2 levels with 10 kJ UV-B (+UV-B+CO_2+T). For each treatment, fifteen replications were maintained per species per SPAR unit.

A humidity and temperature sensor (HMV 70Y, Vaisala, Inc., St. Louis, MO, USA) was used to monitor the relative humidity of each chamber. The monitor was installed in the returning path of airline ducts. A chilled mixture of ethylene glycol and water was injected through the cooling coils located outside the air handler of each chamber via several parallel solenoid valves. The valves opened or closed depending on the cooling requirement to maintain a constant humidity [38].

Pure CO_2 supply was maintained through a compressed gas cylinder using a system that included a pressure regulator, solenoid and needle valves, and a calibrated flow meter [35]. The chamber CO_2 was measured and maintained either at 400 or 720 µmol mol^{-1} with a dedicated infrared gas analyzer (LI-COR, model LI-6252, Lincoln, NE, USA); the drawn gas sample through the lines run underground from SPAR units to the field laboratory building. The sample lines were run through refrigerated water (4 °C) that was automatically drained and through a column of $Mg(ClO_4)_2$ to remove moisture from the gas sample.

The desired elevated UV-B treatment, 10 kJ m^{-2} d^{-1}, was imposed from 12 DAS to the end of the experiment. The square-wave UV-B supplementation systems were used under near-ambient PAR to provide anticipated UV-B radiation dosage. The UV-B radiation was delivered from 0.5 m above the plant canopy for 8 h each day, from 08:00 to 16:00, by eight fluorescent UV-313 lamps (Q-Panel Company, Cleveland, OH, USA) attached horizontally on a metal frame inside each chamber, powered by 40 W variable dimming ballasts. The individual UV lamp was wrapped with solarized 0.07 mm diacetate film to filter UV-C (<280 nm) radiation. The UV-B radiation supplied at the top of the plant canopy was monitored daily at 08:00 with a UVX digital radiometer (UVP Inc., San Gabriel, CA, USA) calibrated against an Optronic Laboratory (Orlando, FL, USA) Model 754 Spectroradiometer, which was used initially to quantify lamp output. The lamp output was adjusted, and the cellulose diacetate films were replaced as needed to maintain the individual UV-B radiation level. The actual biologically effective UV-B radiation was measured in each SPAR chamber at three different locations (in the middle and two corners) to ensure the plants received the exact UV-B dosage of 10 ± 0.18 kJ m^{-2} d^{-1} during the crop growth period.

2.3. Measurements

2.3.1. Phenology and Growth

The total number of leaves (LN) was counted, and plant height (PH) and marketable fresh weight (MFW) were measured on all plants at harvest (26 DAS). Leaf area was measured using the LI-3100 leaf-area meter (LI-COR, Inc., Lincoln, NE, USA). Plant component total dry weights (TD) were measured after oven drying at 75 °C until a constant weight was reached.

2.3.2. Root Image Acquisition and Analysis

Aboveground plant parts were cut and separated from the root systems. Roots were gently washed free of all soil media. The longest root length (LRL) was determined using a ruler. The cleaned individual root systems were floated in 5 mm of water in a 0.4 by 0.3 m Plexiglas tray. A plastic paintbrush was used to untangle and separate roots to minimize root overlap. The tray was placed on top of a specialized dual-scan optical scanner [30] linked to a computer. Gray-scale root images were acquired by setting the parameters to high accuracy (resolution 800 × 800 dpi). Acquired images were analyzed for the total root length (TRL), root surface area (RSA), average root diameter (RAD), root length per volume (RLPV), root volume (RV), number of tips (RT), number of forks (RF), and number of crossings (RC) using WinRHIZO Pro software 2009c [Regent Instruments, Inc., Québec, QC, Canada] [30].

2.4. Data Analysis

2.4.1. Combined Stress Response Index (CSRI)

Based on the summation of relative individual stress responses at each treatment and similar to the cumulative response index quoted in other UV-B studies [32], the combined stress response index (CSRI) was calculated to evaluate the interactive effects of six treatments (+CO_2, +T, +UV-B, +CO_2+T, +CO_2+UV-B, and +CO_2+T+UV-B) in comparison to the control treatment. The CSRI was calculated as the value of a parameter under control (c), subtracted from the value of the parameter under treatment (t), and then by dividing by the value of a parameter under control (c) as follows:

$$\begin{aligned}\mathrm{CSRI} = {} & \frac{(\mathrm{PHt}-\mathrm{PHc})}{(\mathrm{PHc})} + \frac{(\mathrm{LNt}-\mathrm{LNc})}{(\mathrm{LNc})} + \frac{(\mathrm{LAt}-\mathrm{LAc})}{(\mathrm{LAc})} + \frac{(\mathrm{MFWt}-\mathrm{MFWc})}{(\mathrm{MFWc})} + \frac{(\mathrm{ADWt}-\mathrm{ADWc})}{(\mathrm{ADWc})} + \\ & \frac{(\mathrm{RDWt}-\mathrm{RDWc}}{(\mathrm{RDWc})} + \frac{(\mathrm{TDWt}-\mathrm{TDWc})}{(\mathrm{TDWc})} + \frac{(\mathrm{LRLt}-\mathrm{LRLc})}{(\mathrm{LRLc})} + \frac{(\mathrm{TRLt}-\mathrm{TRLc})}{(\mathrm{TRLc})} + \frac{(\mathrm{RSAt}-\mathrm{RSAc})}{(\mathrm{RSAc})} + \\ & \frac{(\mathrm{RADt}-\mathrm{RADc})}{(\mathrm{RADc})} + \frac{(\mathrm{RLPVt}-\mathrm{RLPVc})}{(\mathrm{RLPVc})} + \frac{(\mathrm{RVt}-\mathrm{RVc})}{(\mathrm{RVc})} + \frac{(\mathrm{RTt}-\mathrm{RTc})}{(\mathrm{RTc})} + \frac{(\mathrm{RFt}-\mathrm{RFc})}{(\mathrm{RFc})} + \frac{(\mathrm{RCt}-\mathrm{RCc})}{(\mathrm{RCc})}\end{aligned}$$

where CSRI is the combined stress response index, PH—the plant height, LN—the leaf number, LA—the leaf area of the plant, MFW—the marketable fresh weight, ADW—aboveground dry weight, RDW—the root dry weight, TDW—Total dry weight, LRL—the longest root length, TRL—the total root length, RSA—the root surface area, RAD—the root average diameter, RLPV—the root length per volume, RV—the root volume, RT—the number of root tips, RF—the number of root forks, RC—the number of root crossings under t (treatment) and c (control).

2.4.2. Statistical Analysis

Data were subjected to analysis of variance [40] with a split-plot design considering species and treatment as sources of variance. Replicated values for LN, PH, LA, MFW, ADW, RDW, TDW, LRL, TRL, RSA, RAD, RLPV, RV, RT, RF, and RC were analyzed using one-way ANOVA of the general linear model, PROC GLM, in SAS [40] to determine the effect of multi-stress treatments on the morphological and developmental parameters of kale and mustard. Fisher-protected least significant difference tests at $p = 0.05$ were employed to test the differences among treatments for measured parameters. The standard errors of the mean were calculated and are presented in the figures as error bars.

3. Results

This is the first study providing data for the effects of abiotic multi-stress on the growth and development of roots and shoots of green leafy vegetables of the Brassica family.

3.1. Shoot Growth and Developmental Attributes

3.1.1. Plant Height

Interactive effects of increased CO_2, temperature, and UV-B radiation led to significant differences in plant height (Table 2). Compared to the control treatment, the plants were significantly taller under elevated CO_2 (+CO_2) (8% and 12% in kale and mustard, respectively). Plants grown under elevated temperature conditions along with CO_2 treatment had minimal adverse effects on plant height, showing owing only 12% and 11.7% (+T), and 3.3% and 1.8% (+CO_2+T), reductions in average plant height in kale and mustard, respectively, compared to the control. Plants grown under UV-B conditions alone and elevated temperature and CO_2 produced significantly shorter plants in both Brassica sp., as evident in Figure 1. The greatest plant height reduction was observed under UV-B (53% for kale and 39% for mustard) treatment (Table 3). Among the two Brassica sp., mustard plants were taller than kale plants under all the treatments and showed lesser reductions.

Table 2. The analysis of variance across the treatments of carbon dioxide concentration CO_2, temperature., UV-B radiation, and two crops (kale and mustard), and their interactions on kale and mustard root and shoot growth and developmental traits, plant height (PH), mainstem leaves (LN), whole plant leaf area (LA), marketable fresh weight (MFW), aboveground dry weight (ADW), root dry weight (RDW), total plant dry weight (TDW), the longest root length (LRL), total root length (TRL), root surface area (RSA), average root diameter (RAD), root length per volume (RLPV), root volume (RV), root tips (RT), root forks (RF), and root crossings (RC).

Source of Variance	PH	LN	LA	MFW	ADW	RDW	TDW	LRL	TRL	RSA	RAD	RLPV	RV	RT	RF	RC
Treatment	***	***	***	***	***	***	***	*	***	***	***	***	***	***	***	***
Crop	**	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Trt × Crop	NS	***	***	***	**	**	**	*	**	**	***	**	**	***	**	**

*** indicates significance levels, **, *, and NS, representing $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p > 0.05$, respectively.

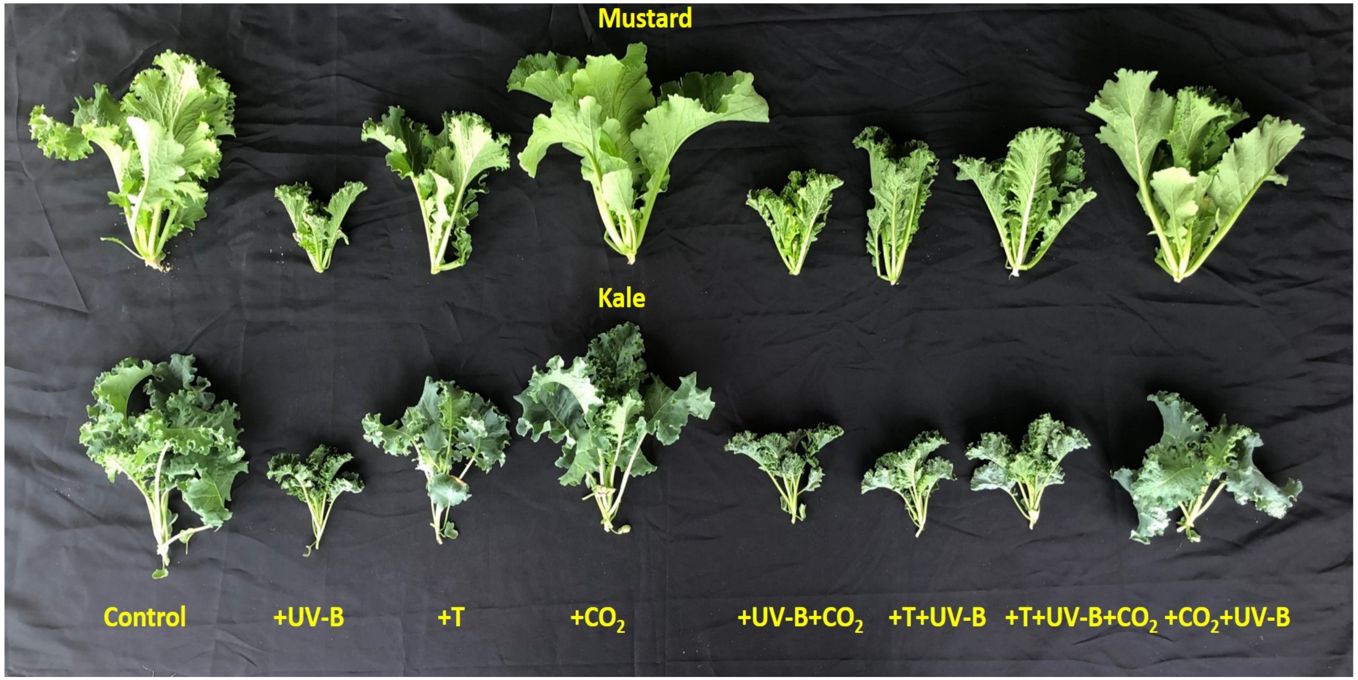

Figure 1. A pictorial presentation of the impact of CO_2 concentration (control, 400 µmol mol^{-1} and +CO_2, 720 µmol mol^{-1}), elevated temperatures (25/17 °C and 35/27 °C (day/night)), and radiation (control, 0 and +UV-B, 10 kJ m^{-2} d^{-1}), and their interactions on the morphological growth of kale and mustard.

Table 3. Mean values and percent change for plant height (PH), leaf number (LN), leaf area (LA), marketable fresh weight (MFW), aboveground dry weight (ADW), root dry weight (RDW), and total dry weight (TDW) measured under CO_2 concentration (control, 400 µmol mol^{-1} and +CO_2, 720 µmol mol^{-1}), elevated temperatures (25/17 °C and 35/27 °C (day/night)), and UV-B radiation (control, 0 kJ m^{-2} d^{-1}, and +UV-B, 10 kJ m^{-2} d^{-1}), and their interactions for kale and mustard at 26 DAS.

	Traits	Crop	Treatments						
			Control	**0**	**+T**	**+UV-B**	**+T+CO_2**	**+UV-B+CO_2**	**+UV-B+CO_2+T**
	PH (cm plant^{-1})	Kale	30.8	33.3 (+8%)	27.2 (−11.7%)	14.3 (−53%)	29.8 (−3.3%)	17.3 (−43.8%)	18.5 (−40%)
		Mustard	31.9	35.8 (+12%)	28 (−12%)	19.3 (−39%)	31.3 (−1.8%)	19.5 (−38.8%)	22.3 (−30%)
	LN (plant^{-1})	Kale	9.33	9.7 (+3.9%)	8.7 (−8.8%)	9.5 (+1.8%)	9 (−3.5%)	17.3 (−43.8%)	18.5 (−40%)
		Mustard	11.7	12.7 (+8.5%)	9.7 (+17%)	10.8 (−7.6%)	15.8 (+35%)	19.5 (−38.8%)	22.3 (−30%)
	LA (cm^2 plant^{-1})	Kale	600.5	779.3 (+29.8%)	424.5 (−29.3%)	131.7 (−78%)	565.7 (−5.7%)	226.3 (−62.3%)	292.9 (−51.2%)
Shoot Traits		Mustard	1305.1	1629.6 (+24.8%)	983.9 (−24.6%)	363.5 (−72%)	1495.5 (−8.2%)	380.5 (−70.8%)	653.5 (−50%)
	MFW (g plant^{-1})	Kale	42	51.5 (+22.7%)	24 (−42.7%)	12.7 (−69.7%)	35 (−16.9%)	20.9 (−50%)	22.5 (−46.3%)
		Mustard	76.3	116.2 (+52%)	61.3 (−19.7%)	30.4 (−60.2%)	96.9 (26.8%)	30.6 (−60%)	50 (−34.5%)
	ADW (g plant^{-1})	Kale	3.69	5.7 (+55%)	2.8 (−24.6%)	1.3 (−64%)	4.2 (+13.7%)	2.6 (−28.4%)	2.7 (−27.6%)
		Mustard	5.93	8.8 (+48%)	5.4 (−9.5%)	2.6 (−56.8%)	8.6 (+45.7%)	2.7 (−54.5%)	4.6 (−22.4%)
	RDW (g plant^{-1})	Kale	0.37	0.6 (+55.2%)	0.3 (−30%)	0.1 (−63%)	0.4 (0%)	0.3 (−65.6%)	0.3 (−20%)
Dry weight traits		Mustard	0.79	1 (+32.8%)	0.7 (−5.9%)	0.4 (−52.7%)	1.1 (+43.4%)	0.3 (−60%)	0.7 (−7%)
	TDW (g plant^{-1})	Kale	4.05	6.3 (+55.3%)	3 (−25%)	1.5 (−64%)	4.6 (+12.5%)	2.9 (−28%)	3 (−27%)
		Mustard	6.71	9.9 (+47%)	6.1 (−9%)	2.9 (−60%)	9.8 (+45.6%)	3 (−55%)	5.3 (−20%)

3.1.2. Leaf Number

The number of leaves produced in the plants under increased CO_2, UV-B, and temperature treatments was significant in the present study (Table 2). The crops showed different responses under different treatments for the number of leaves produced during the treatment (Figure 1). Fewer leaves in mustard were observed under +UV-B+CO_2 treatment, where the reduction was 25% compared to the control (Table 3; Figure 1). In the case of kale, the maximum deduction was observed under high-temperature treatment. More leaves were observed under +CO_2 and high temperatures and UV-B (+CO_2+T+UV-B) in both the crops (4% and 8.5% at +CO_2; 9% and 4.2% at +CO_2+T+UV-B in kale and mustard, respectively). A maximum increase in the number of leaves was recorded at high UV-B and CO_2 (+CO_2+UV-B) for kale (12.5%) and high temperature and CO_2 (+T+CO_2) for mustard (35%).

3.1.3. Leaf Area

The leaf area exhibited significant differences under all the treatments. Higher leaf area was recorded under +CO_2 treatment, as clearly noticeable from Figure 1 (30% in kale and 25% in mustard), compared to the control (Table 3; Figure 2A.). The highest reduction in leaf area in both crops was observed under the UV-B treatment alone (+UV-B), 78% (kale), and 72% (mustard) compared to their respective controls. Elevated CO_2 (+CO_2) seemed to alleviate the adverse effects of high temperature (+T) on leaf area leading to the most negligible reduction in both crops (5.7% and 8.2% in kale and mustard, respectively).

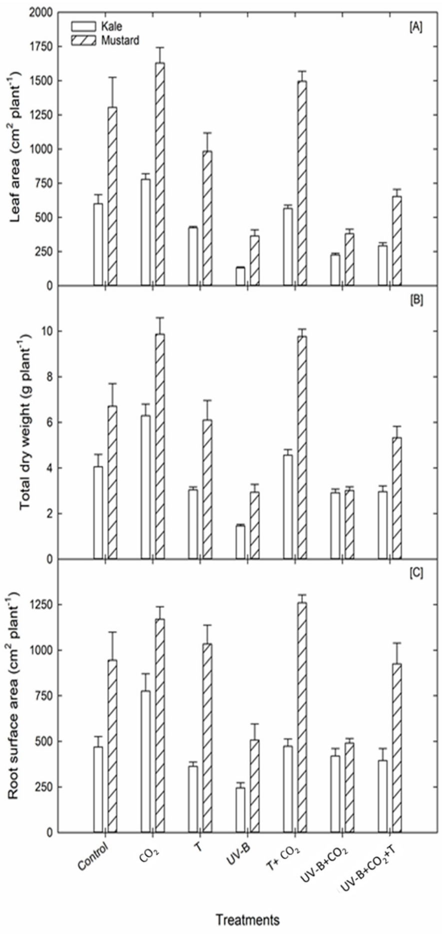

Figure 2. Impact of CO_2 concentration (control, 400 μmol mol^{-1} and +CO_2, 720 μmol mol^{-1}), elevated temperatures (25/17 °C and 35/27 °C (day/night)), and UV-B radiation (control, 0 and +UV-B, 10 kJ m^{-2} d^{-1}), and their interactions on (**A**) leaf area, (**B**) total dry weight and (**C**) root surface area for kale and mustard. Bars indicate standard errors of the mean.

3.1.4. Marketable Fresh Weight

Marketable fresh weight, which determines the economic value of green leafy vegetables, decreased significantly under all the treatments except for the +CO_2 treatment. The average marketable fresh weight ranged from 12.7 to 116.2 g plant^{-1}, with the lowest value under +UV-B treatment and the highest under +CO_2 treatment alone (Figures 1 and 3; Table 3). Accordingly, the marketable fresh weight decreased by 46.3% and 34.5% in kale and mustard under the +UV-B+CO_2+T treatment. The marketable fresh weight doubled in mustard under the CO_2 treatment compared to its control counterparts, whereas an increase of 23% was recorded in kale.

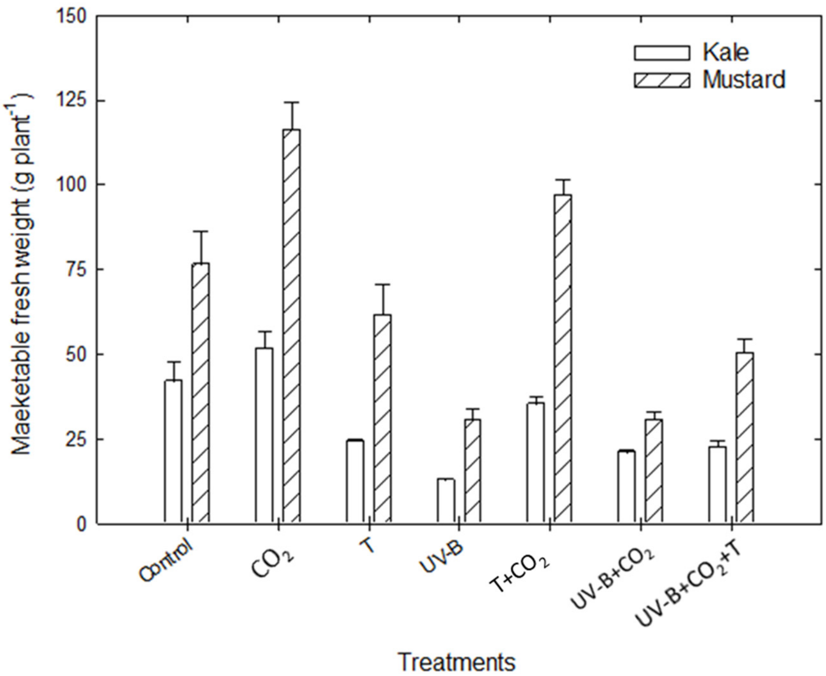

Figure 3. Impact of CO_2 concentration (control, 400 µmolmol^{-1} and +CO_2, 720 µmol mol^{-1}), elevated temperatures (25/17 °C and 35/27 °C (day/night)), and UV-B radiation (control, 0 and +UV-B, 10 kJ m^{-2} d^{-1}), and their interactions on marketable fresh weight for kale and mustard. Bars indicate standard errors of the mean.

3.1.5. Dry Weight Components

Like marketable fresh weight, significant reductions in aboveground dry weight, accounting for 64% (kale) and 57% (mustard) of the decrease compared to their control, were observed under +UV-B treatments. The CO_2 treatment alone (+CO_2) and in combination with elevated temperature (+T+CO_2) recorded an increase of 55% (kale) and 48% (mustard), and of 14% (kale) and 46% (mustard), in aboveground dry weight, respectively, compared to their control treatments (Table 3). Unlike other shoot parameters, the highest reduction in root dry weight was observed under the elevated UV-B and CO_2 treatment (+UV-B+CO_2), which was 66% and 60% in kale and mustard, respectively, compared to their respective controls.

The minimum adverse effect on root dry weight in kale was observed under the +UV-B+T+CO_2 treatment with an average decrease of 20%. In mustard, the minimum decrease was observed under the temperature treatment alone (6%) in comparison to the control. Under the CO_2 treatment and elevated temperature (+T+CO_2), there was no change in the root dry weight of kale, whereas an increase of 43.4% was observed in mustard.

The total dry weight produced during the experimental period ranged from 1.46 to 9.87 g plant^{-1} (Table 3, Figure 2B); plants grown under +UV-B registered the lowest while plants grown under +CO_2 treatment alone showed the greatest total dry weight in both the crops. All the stresses, either alone or in combination, caused significant differences in total dry weight. The reductions in total dry weight under +UV-B, +T+UV-B+CO_2, and +UV-B+CO_2+T treatments were 64% and 60%; 25% and 9%; 28% and 55%; and 27% and 20%, in kale and mustard, respectively, when compared to the control. The total dry weight in both crops increased under the +CO_2 treatment and the +T+CO_2 treatment. Among the two crops, mustard had the higher total dry weight under all the treatments.

3.2. Root Growth and Developmental Attributes

3.2.1. Root Growth Traits

All the stress treatments alone or together led to significant differences in the root length of both crops. Both longest root length (LRL; 24% in kale and 17% in mustard) and total root length (TRL; 43% in kale and 39% in mustard) showed the highest reduction under the elevated UV-B treatment. The longest root length in kale decreased under all treatments except for +CO_2 treatment (14.7%) and +UV-B+CO_2 treatment (1.73%). In contrast, in mustard, LRL increased under all the treatments except +UV-B and +UV-B+CO_2 (3.2%), compared to their respective control (Table 4). The minimum adverse effect on total root length was observed under +UV-B+CO_2 treatment in the case of kale, with an average decrease of 1%. In mustard, the most negligible reduction of 1% each was observed under +CO_2 treatment and the CO_2 treatment, together with UV-B and elevated temperature (+UV-B+CO_2+T). Compared to the controls, TRL values increased for both crops under CO_2 treatment combined with high temperature (+T+CO_2).

Root surface area (RSA), average root diameter (RAD), root length per volume (RLPV), and root volume (RV) decreased significantly under +UV-B treatment, +UV-B+CO_2 treatment, and all three stresses together in both the crops. The +CO_2, alone or in combination with elevated temperature (+CO_2+T), exhibited an increase in RSA and RV compared to their control treatments in both crops (Table 4; Figure 2C). RSA and RLPV values for kale decreased by 22.6% and 12.6%, respectively, under the elevated temperature, whereas increases of 9.4% and 22.6% were observed in mustard. RAD and RV values for both crops decreased under high-temperature conditions.

3.2.2. Root Developmental Traits

All the root developmental traits showed significant reductions, accounting for 25% and 28% of the decline in the number of root tips (RT), 52% and 44% of the decrease in the number of root forks (RF), and 41.6%, and 37.5% of the decline in the number of root crossings (RC), in kale and mustard respectively, compared to their control under the +UV-B treatment. The +CO_2 treatment exhibited an 82% (kale) and 19.3% (mustard) increase in the number of root forks concerning its control treatment. Although the CO_2 treatment alone positively impacted the number of root tips and crossings in kale, it decreased both parameters in mustard. Under the high-temperature conditions, all the developmental traits fell (28% in RT, 25.3% in RF, and 6% in RC) in kale but showed an increase in mustard (4.6% in RT, 20% in RF, and 42.5% in RC; Table 4). Under the combination of all three stresses (+UV-B+CO_2+T), a decrease in the number of root tips and root forks in both crops and root crossings in kale was recorded; however, a slight increase (0.5%) was observed in the case of mustard.

Table 4. Mean values and percent change for the longest root length (LRL), total root length (TRL), root surface area (RSA), average root diameter (RAD), root length per volume (RLPV), root volume (RV), number of root tips (RT), number of root forks (RF) and number of root crossings (RC) measured under CO_2 concentration (control, 400 µmol mol^{-1} and +CO_2, 720 µmol mol^{-1}), elevated temperatures (25/17 °C and 35/27 °C (day/night)), and UV-B radiation (control, 0 kJ m^{-2} d^{-1}, and +UV-B, 10 kJ m^{-2} d^{-1}), and their interactions for kale and mustard at 26 DAS.

Root Traits	**Crop**	**Treatments**						
		Control	**+CO_2**	**+T**	**+UV-B**	**+T+CO_2**	**+UV-B+CO_2**	**+UV-B+CO_2+T**
LRL (cm plant^{-1})	Kale	38.3	44 (+14.7%)	31.5 (−17.8%)	29 (−24.3%)	29.8 (−22%)	39 (+1.7%)	31.7 (−17.3%)
	Mustard	41.2	42.7 (+3.6%)	48.2 (+17%)	34.2 (−17%)	42 (+2%)	39.8 (−3.2%)	45.7 (+10.9%)
TRL (cm plant^{-1})	Kale	3389	5327.1 (+57%)	2961.7 (−12.6%)	1924.7 (−43%)	3443.1 (+1.6%)	3352.3 (−1%)	2953.5 (−13%)
	Mustard	5717.9	5659.8 (−1%)	7012 (+22.6%)	3477.3 (−39%)	6011.6 (+5%)	3626 (−36.5%)	5665.1 (−1%)
RSA (cm^2 plant^{-1})	Kale	469.1	775.1 (+65%)	362.9 (−22.6%)	244.9 (−47.7%)	473 (+1%)	419.5 (−10.5%)	394.7 (−15.8%)
	Mustard	944.8	1169.9 (+24%)	1033.7 (+9.4%)	507.3 (−46.3%)	1259.2 (+33%)	491 (−48%)	924.6 (−2%)
RAD (mm plant^{-1})	Kale	0.4	0.5 (+4.3%)	0.4 (−11%)	0.4 (−7%)	0.4 (−1%)	0.4 (−9.2%)	0.4 (−1%)
	Mustard	0.5	0.7 (+28.7%)	0.5 (−10%)	0.4 (−12%)	0.7 (+30%)	0.4 (−16.5%)	0.5 (−1%)
RLPV (cm m^{-3})	Kale	3389	5327.1 (+57%)	2961.7 (−12.6%)	1924.7 (−43%)	3443.1 (+1.5%)	3352.3 (−1%)	2953.5 (−13%)
	Mustard	5717.9	5659.8 (−1%)	7012 (+22.6%)	3477.3 (−39%)	6011.6 (+5%)	3626 (−36.5%)	5655.1 (−1%)
RV (cm^3 plant^{-1})	Kale	5.2	9.1 (+75%)	3.5 (−31.5%)	2.5 (−52%)	5.2 (+0.6%)	4.2 (−19%)	4.2 (−19%)
	Mustard	12.5	19.9 (+58%)	12.2 (−2.5%)	5.9 (−52.5%)	21.1 (+68%)	5.3 (−57.4%)	12.1 (−3.3%)
RT (no. plant^{-1})	Kale	10,272.3	12,891.5 (+25.4%)	7382 (−28%)	7738.5 (−24.6%)	6579.3 (−36%)	7855.2 (−23.5%)	6836.8(−33.4%)
	Mustard	14,876	12,958.2 (−13%)	15,573.3 (+4.6%)	10,666.7 (−28%)	11,595.7 (−22%)	9788.2 (−34%)	11,546.1(−22.3%)
RF (no. plant^{-1})	Kale	31,897.8	12,891.5 (+25.4%)	23,822.2 (−25.3%)	15,334.3 (−52%)	31,979 (−0.3%)	30,935.3 (−3%)	24,168 (−24%)
	Mustard	64,775.2	77,329.1 (+19.3%)	77,665.8 (+20%)	36,247.7 (−44%)	83,101.7 (+28.3%)	37,047.7 (−43%)	62,078.5 (−4%)
RC (no. plant^{-1})	Kale	2791.2	4958.2 (+77.6%)	2619.8 (−6%)	1628.2 (−41.6%)	3037.5 (+9%)	3403.3 (+22%)	2380.6 (−14.7%)
	Mustard	4531	4387.2 (−3%)	6458.2 (+42.5%)	2830.2 (−37.5%)	4713.3 (+4%)	3436.7 (−24%)	4552. 83 (+0.5%)

3.3. Combined Stress Response Indices (CSRI)

The combined stress response index is the sum of relative individual stress responses at each treatment. CSRI values ranged from −7.4 to 8.3 in kale and −6.8 to 3.7 in mustard. The lowest CSRI values for both crops were observed under +UV-B treatment, suggesting higher deleterious effects of UV-B treatment on all the parameters (Figure 4). In comparison, the highest value for kale was observed under +CO_2 treatment and for mustard under +T+CO_2 treatment, pointing towards the positive impacts of elevated CO_2 concentrations. CSRI values under all the treatments except +CO_2 treatment and its combination with high temperature (+CO_2+T) were negative in kale. However, in mustard, CSRI values under +UV-B, +UV-B+CO_2 treatment, and under all the three stress together (+UV-B+CO_2+T) were negative (Figure 4).

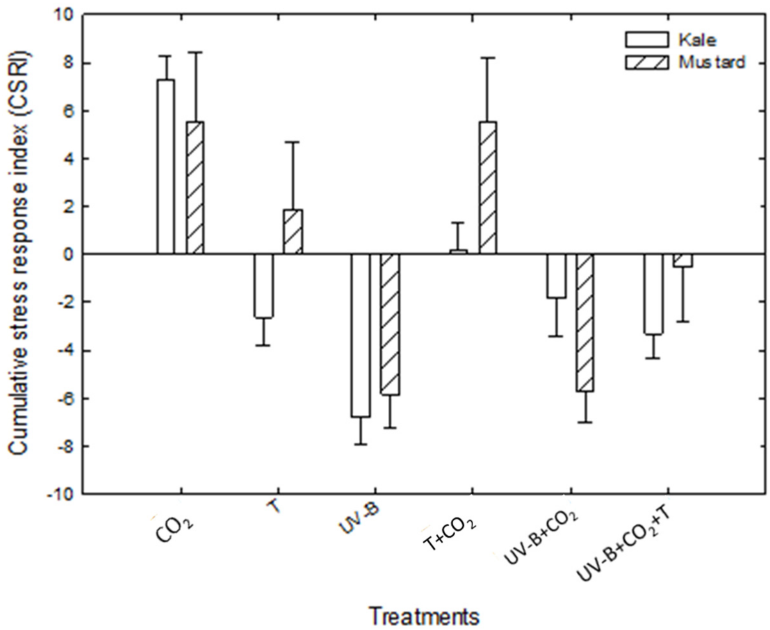

Figure 4. Cumulative stress response index (CSRI) calculated over all the treatments of kale and mustard in response to elevated carbon dioxide (720 ppm) (+CO_2), high temperature (35/27 °C, day/night) (+T), and increased UV-B radiation (10 kJ m^{-2} d^{-1}) (+UV-B) and their interactions.

4. Discussion

Brassica plants are often exposed to multiple stresses co-occurring during their growing season. Thus, it is imperative to conduct experiments in growth chambers in an environment that mimics natural conditions. In the current experiment, growing plants in SPAR chambers under fully controlled conditions permitted us to identify the functional relationships of growth and developmental responses of kale and mustard plants in response to the interaction of multiple abiotic stresses similar to those under natural conditions. Thus, this information may be helpful in management decisions and for crop model improvements to stimulate the vegetative growth of these crops in the field environment.

UV-B and mostly elevated temperatures drastically affect crop shoot, root growth, and developmental traits. However, CO_2 masked most of the other stresses' adverse effects (Tables 3 and 4). Plant height, leaf number, leaf area, and dry weight traits were affected mainly by UV-B stresses alone or combined with the other two stresses. Reduction in plant height under higher UV-B levels has been recently reported in *Capsicum annuum* [41,42] and *Brassica napus*. The shorter plants may be due to specific photomorphogenic responses of plants to elevated UV-B radiation via a UV-B photoreceptor [43]. Moreover, low photosynthetically active radiation (PAR, 400–700 nm) may have also affected the plant. It has been shown that increased PAR decreases the impacts of UV-B radiation on plant height [44]. Reduced plant height was also observed in two other brassica species

(*B. rapa* and *B. nigra*) exposed to UV-B radiation [45]. Studies on crops such as *Vigna mungo*, *V. radiata*, and *Glycine max* [46–48], *Triticum aestivum* and *Amaranthus tricolor* [49], and *Oryza sativa* [50] have shown retarded growth and reduced leaf area expansion in response to UV-B radiations [51]. The most likely reason for the reduction in growth is direct damage to DNA [52]. It has been observed that a plant under heat stress can delay cell division through reduced cell elongation. This affects the shoot net assimilation rates and the plant's total dry weight, ultimately reducing plant growth [53]. Heat stress also decreases stem growth, resulting in reduced plant height [54]. Following our results, similar reductions in plant height at higher temperatures have been reported in other crops, including recent reports in *Brassica juncea* [55] and *Oryza sativa* [56].

The results of a reduction in major growth and developmental parameters, such as total leaf area and fresh and dry weights, number of leaves, and height of plants (Table 3), obtained in this study under higher UV-B levels corroborate those found in *Arabidopsis thaliana* leaves [57], soybean [33], cotton [23], maize [27,58], *Phaseolus vulgaris* [59] and sweet potato [60]. One of the most common responses to UV-B is a leaf area decrease because of a reduction in cell division and expansion [61–63]. The decline in leaf area associated with the lower concentration of photosynthetic pigments seems to be the cause of the decrease in growth and the reduction in stem length and root dry mass as recorded under UV-B radiation treatment in *P. vulgaris.* These factors can result in lower absorption of sunlight and affect photosynthetic activity, leading to a decrease in photosynthesis, indirectly affecting plant growth [63]. In line with our findings, growth reduction under UV-B has also been reported in *Arabidopsis thaliana* [64] and *Capsicum annuum* [42]. In contrast, Nedunchezhian and Kulandaivelu [65] reported that slightly elevated UV-B radiation increases leaf area in cowpea. An increase in plant height, leaf area, and dry weight under elevated UV-B was also reported in *Ocimum basilicum* [66]. Leaf area reduction in rice has been recently reported under high temperatures alone and with elevated CO_2 [67]. High temperature and UV-B interaction decreased leaf area in *Brassica napus* [68].

The horticultural crops having a C_3 photosynthetic metabolism have shown beneficial effects indicating the increase in growth traits in onion [69,70] and tomato at 550 ppm CO_2 [71]. In perennial crops such as coconut (*Cocos nucifera* L.), studies indicate an increase in shoot height, leaf area, and shoot dry weight due to elevated CO_2 of up to 36% over chamber control [72,73]. We found all the shoot and root parameters increased under high CO_2 levels in both crops.

Approximately 20% of crops are sensitive to UV-B radiation regarding dry mass reduction [60,74]. We observed a decrease in marketable fresh weight and dry weight traits under all the treatments except CO_2 alone in the present study. A reaction to stress caused by UV-B radiation in plant development and metabolism could explain the reduction in dry and fresh leaf mass [75]. Fresh and dry weight reductions by 10–12% were reported in *Beta vulgaris* under UV-B [76]. Dai et al. [77] reported that, after a few weeks of UV-B exposure, the plant dry weight of rice was significantly reduced. Zuk-Golaszewska et al. [78] also reported a decrease in dry weight under high UV-B levels in *Avenafatua* and *Setariaviridis*.

On the contrary, a different response was found on broad bean and wheat, in which the plant's dry mass increased with the rising UV-B [79]. Like studies on brood bean and wheat, Zhang et al. [80] also reported an increase in whole plant dry weight in *Prunella vulgaris* plants when exposed to 15-day UV-B radiations in a growth chamber. This suggests that the UV-B effect is species/cultivar specific, and sometimes it benefits the growth and development of plants [81]. An increase in total dry weight was also observed in *Ocimum basilicum* and *Mentha piperita* under elevated temperatures [82]. Reduced plant weight under high temperature can also be related to decreased photosynthesis, increased transpiration [83], and, in turn, reduced water use efficiency [84]. Like our study under elevated temperatures, a decrease in dry weight has been recently reported in three *Brassica* sp. [85], *Brassica oleracea* [86], *Raphanus sativus* [87], and *Chenopodium quinoa* [88]. High temperature and UV-B interaction decreased leaf weight in *Brassica napus* [68]. Interaction of elevated temperatures and CO_2 increased plant height, the number of leaves, and the leaf area in *Fragaria × ananassa* [89], *Capsicum annuum* [90], and *Solanum lycopersicum* [91]. In contrast to our results, high temperature and elevated CO_2 reduced the whole-plant dry weight in rice [67].

The different effects of UV-B radiation and CO_2 on plant growth and development have been extensively studied for a wide range of horticultural and agronomic crops. Still, little work has considered UV-B and CO_2 interaction [41,92–97]. In an experiment conducted by Teramura et al. [92], soybean, wheat, and rice grown under two levels of CO_2 and UV-B showed increased total plant biomass in all three species under elevated CO_2. However, under the interaction of elevated CO_2 with enhanced UV-B radiation, these effects were eliminated in wheat and rice but remained in soybean. This indicates that the combined effects of UV-B and CO_2 are species-specific, and that UV-B can modify the positive effects of CO_2. Moreover, Ziska and Teramura [98] with rice, and Van de Staaij et al. [99] with wild ryegrass, have shown that the effects of CO_2 and UV-B radiation are independent. Our study revealed that elevated CO_2 can partially alleviate some of the adverse effects of UV-B radiation in *Brassica* sp. Similar results were observed by Brand et al. [100] in cotton. Qaderi et al. [101] experimented on the interactive effects of high temperature and UV-B levels in *Brassica napus*. They observed that the higher temperature with enhanced UVB negatively affected all the growth traits. These results confirm our findings.

Understanding root responses to changes in the aerial environment is essential in deciphering the crop responses to predicted climate changes [100]. Little is known about the effects of abiotics on the root system of kale and mustard compared to other major crops such as corn, rice, and cotton in the US Midsouth during seedling growth [102–104]. In the present study, elevated CO_2 concentration stimulated root growth, whereas high temperature and +UV-B either individually or in combination suppressed most root traits. Previous root studies on sorghum [105] and tomato [106] have reported significant root diameter, root volume, root length, and dry weight density under elevated CO_2 concentrations. Many studies have extensively documented the responses of plants to increasing atmospheric CO_2. However, the effects of elevated CO_2 on root dynamics have not been explored much, despite their importance for global carbon budgets and nutrient cycling in ecosystems. Therefore, the current data on the effects of elevated atmospheric CO_2 levels on root dynamics are insufficient to draw any conclusions. Different studies indicated a general increase in root growth under elevated CO_2 compared to ambient CO_2 levels [107,108].

In an experiment conducted by Sindhøj et al. [109], increased root growth was observed in nutrient-poor semi-natural grassland at an elevated CO_2 concentration. Moreover, elevated CO_2 affected root architecture through increased branching compared to ambient CO_2 in a shortgrass steppe. Root length, the number of roots, and the diameter of roots in the upper soil profile were also greater under such conditions [108]. In contrast to our findings, Ostonen et al. [110] reported decreased specific root lengths under elevated levels of CO_2.

Total root length, RSA, and RAD have been used to characterize root systems and evaluate their functional size [111]. These characteristics help predict nutrient uptake ability and performance under stress conditions. The root systems of kale and mustard at early developmental stages have not been adequately characterized. The present study investigated root structural parameters, including root development concerning RL, RCL, RSA, RV, RAD, root distribution pattern in the soil column, RS, and root branching. UV-B treatment alone caused significant reductions in all the root growth parameters. Reductions in root length under elevated temperatures have also been observed in *Solanum lycopersicum*, *Cucumis sativus,* and *Solanum melongena* [112].

Extreme temperatures have been observed to profoundly impact the growth and development of plant root systems (Table 4). The well-documented relationships between extreme temperatures and specific plant functions include nutrient uptake, photosynthesis, and carbon partitioning [29]. Elevated temperatures appear to impact mustard roots positively but led to a decrease in all root parameters in kale. Like our results in kale, Choi et al. [87] reported a reduction in root length and diameter under high temperatures in *Raphanus sativus* and *Brassica campestris*. A significant decrease in root parameters under high temperatures has also been reported in canola [113]. RLPV is directly related to the plants' water uptake ability because water is mainly absorbed passively, and generally reflects the development of lateral roots [114]. Plant roots optimize their root architecture to acquire water and essential nutrients. The number of root tips, forks, and crossings plays a vitally important role in the root architecture of a plant. They can enhance penetration through soil layers, ultimately leading to a positive effect on plant nutrient uptake. In the present study, root tips, forks, and crossings decreased significantly under UV-B treatment alone and together with elevated temperature and CO_2 treatment, indicating the harmful effects of multiple stresses on root architecture.

Although the adverse effects of CO_2 and the increase in other greenhouse gases in the environment, which appear to be a cause of global warming, are a global concern, elevated CO_2 may positively affect plants by mitigating the detrimental effects caused by UV-B radiation. Generally, high levels of atmospheric CO_2 have shown beneficial effects on plants, whereas enhanced levels of UV-B radiation are detrimental [115]. However, the relationship between these and other environmental factors, including temperature, light, drought, and salinity, is complex and has not been studied much. Therefore, multifactorial experiments must be undertaken to have a better understanding of plant growth and physiological responses to environmental stressors.

5. Conclusions

In the present study, the interactive effects of elevated CO_2, temperature, and UV-B radiation on the development of two *Brassica* sp. were quantified under a sunlit environment, similar to field conditions under optimum nutrient conditions. Plants grown at +UV-B alone or elevated temperatures produced shorter plants, with smaller leaf areas and shorter roots and reduced biomass. Elevated UV-B conditions had significant adverse effects on most shoot and root parameters, whereas $+CO_2$ resulted in an increase in all vegetative traits. The current study results indicate that high temperature and +UV-B may be major abiotic stressors that impact kale and mustard growth and development during the early season. Kale and mustard are some of the oldest green leafy vegetables globally, known for their health benefits; however, not much information is available on the interactions of various abiotic stresses in these two crops. Therefore, more research is required in this arena to better understand these two *Brassica* species. Moreover, improving early seedling growth and developmental response to abiotic stresses would benefit the current environment. Such improvements will be more apparent for vigorous plant growth in future projected climates.

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