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RESEARCH ARTICLE

Effect of water stress and enhanced solar UV-B radiation (280-320 nm) on physiology, biochemical and antioxidant activity in *Raphanus sativus* L.

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Abstract

Multiple stress factors are produced in the natural ecosystem as a result of global climatic shifts. The two most significant abiotic stress factors that have an impact on plant development are UV-B radiation and water stress. In this study, long-term UV-B radiation (4 hr/day) and well water conditions were applied to *Raphanus sativus* L. seedlings. Each unfavourable circumstance has a different effect on the plants' physiological and biochemical mechanisms. There are numerous ways the plant reacts to stress, including improved antioxidant activity, stress avoidance and increased proline content. The photosynthetic pigment study found that, compared to control and combined stress, the individual stress increased the production of chlorophyll and carotenoids. The levels of anthocyanin and flavonoids in *R. sativus* plants were significantly affected by UV-B and drought when water stress and UV-B were employed independently. With increasing and decreasing chlorophyll pigment content, linked stress action on non-photosynthetic chemicals was more likely to occur. Proline levels were higher in water-stressed *R. sativus* plants. The particular activities of antioxidant enzymes raised the likelihood of plants succumbing to UV-B alone and water stress plants more than when both stress elements were applied together. Further research is necessary to understand the mechanisms that regulate *R. sativus* development in response to UV-B and drought stress, as well as the interactions between osmotic potentials, plant water and the activation of stress-associated proteins and adaptive osmolytes.

Keywords: antioxidant; chlorophyll; drought; proline; *Raphanus sativus*; UV-B

Introduction

Light is a vital component of photosynthesis, but along with it comes exposure to ultraviolet-B (UV-B) radiation (280-315 nm), a component of sunlight that affects plant growth and physiology. While UV-B radiation reaching the Earths' surface is limited, its effects include reduced growth, breakdown of photosynthetic pigments, decreased carbon assimilation, altered biomass allocation and an overall reduction in plant biomass (1). In addition to UV-B radiation, drought stress significantly impacts crop growth, yield quality and quantity. Drought causes reduced growth rates, inhibited stem elongation, diminished stomatal activity and restricted leaf expansion (2). The combined impact of these stressors-UV-B and drought-presents a unique challenge for plants, often amplifying oxidative stress and resulting in substantial DNA damage, decreased photosynthetic efficiency, reduced water use efficiency and diminished biomass yield (3).

Understanding how these stresses interact is crucial for deciphering plant adaptation to changing climatic conditions, especially given the uncertainty around mechanisms that determine crop sensitivity or resistance to combined stresses. Both UV-B and drought stress lead to an overproduction of Reactive Oxygen Species (ROS), which can disrupt cellular homeostasis. Excessive ROS levels cause oxidative damage, altering plant metabolism and resulting in cellular dysfunction (4). Plants counteract these effects using a robust antioxidant defence system, which includes enzymatic and non-enzymatic components distributed across various subcellular compartments. This system helps maintain ROS equilibrium, as excessive ROS loss could impair intracellular signalling pathways (5).

Additionally, plants employ osmoprotectants like proline, which stabilise cell membranes and proteins under water stress and mitigate oxidative damage. Proline is particularly notable for its significant accumulation in response to drought stress, acting as an effective marker for drought damage and a potential stress tolerance mechanism (6). In crops such as maize and beans, proline levels in shoots and roots increase markedly during drought, underscoring its role in combating water stress (7). This study investigates how *Raphanus sativus* L. (radish) seedlings respond to water stress and UV-B radiation by examining photosynthetic pigment dynamics, antioxidant enzyme activities, secondary metabolite profiles and overall plant defence mechanisms. The findings aim to elucidate the interplay between UV-B and drought stress, providing insights into plant adaptability and strategies for mitigating combined abiotic stress effects.

Material and Methods

Plant growth and experimental treatment

Trial plots will display certified *Raphanus sativus* L. seeds obtained from Farm Aid in Madurai. When watering the plants often, precautions are required to prevent microbial or pest infestation during the initial stage. The treatment will be applied to plants that are in the initial foliage leaf phase.

At 5 days old, the seedling received the following care: one group of plants flourished in the presence of sun radiation, on alternating days, the second group of plants accumulated water stress, third group of plants were artificially exposed for 4 hr to 20 % UV-B radiation using Philips TL40W/12 sunlamps (Philips, Holland) cantered at solar noon and water stress and + 20 % UV-B radiation were both obtained by the fourth set of plants.

Growth measurement of a plant

When the seedlings are uprooted, the issues with shoot length and fresh weight will be immediately remedied. After drying the plants for 24 hr at 90 °C, the dry weight of the plants is controlled.

Pigments measurement

The amount of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids was determined after pigments were extracted in 80 % acetone (8).

Carotenoids measurement

The 80 % acetone extracts' total carotenoids were estimated using the absorbance at 480 nm. To be precise, the chlorophyll intervention was calculated using Eqn. 1 (9).

$$\text{Carotenoids (mg/L)} = \frac{1000\,A_{470} - (3.27\,Chl\,a) - (104\,Chl\,b)}{229} \times 100 \quad \text{(Eqn. 1)}$$

Flavonoids determination

Using 5 mL of 80 % acidified methanol, fresh leaf samples weighing 100 mg were broken into tiny pieces and incubated for the entirety of the night at 4 °C in the dark (80:20:1). Following centrifugation to remove debris, the flavonoid concentration was determined to be A units/g of fresh leaf weight by reading the absorbance at 315 nm (10).

Determination of anthocyanins

To extract the anthocyanins, the leaves were crushed in methanol that had been 80 % acidified (80:20:1 of methanol:water:HCl). After centrifugation, the apparent extract was utilised to measure the anthocyanin concentration by measuring the absorbance at 530 and 657 nm (11).

$$\text{A/g fresh weight} = (A_{530}) - (0.3 \times A_{657}) \quad \text{(Eqn. 2)}$$

DPPH radical scavenging assay

With a few adjustments to the earlier approach described, the ability of each component to scavenge radicals at various concentrations was evaluated (12). The effective relative concentration (EC_{50}), defined as mg of extract per μmol^{-1} of DPPH radical, was computed using the equation below. At this concentration, half of the DPPH radical has been detached. The calibration curve (y=1.145E-2×-4.192E-3, r=0.9999, where y = absorbance and × = concentration of DPPH) at 514 nm was employed to establish the reaction systems' equally unique DPPH concentration (92.18 µmol L^{-1}). Every analysis was completed in duplicate. A Thermo Scientific Evolution 60S UV-Visible spectrophotometer was used to gather all spectrophotometric data.

Hydrogen peroxide scavenging assay

By macerating 100 mg of fresh tissue in 0.1 % trichloroacetic acid (2 mL, TCA), hydrogen peroxide levels were reduced. Supernatant from the homogenate was combined with 10 mM potassium phosphate buffer and 1 M potassium iodide in 0.5 mL, after the homogenate had been centrifuged at 12000 g for 15 min (1 mL). At 390 nm, absorbance was resolved (13).

Proline determination

Proline content was ascertained by using the standard procedure (14). Sample leaves (0.2 g) were homogenised with 3 mL of sulphosalicylic acid (3 % w/v) in a mortar and pestle. The homogenate was then centrifuged at 18000 g for 15 min. After that, in a test tube, the supernatant was diluted with 2 mL of freshly diluted acid ninhydrin solution and 2 mL of glacial acetic acid. Before being allowed to cool to room temperature, tubes were incubated at 100 °C in a water bath for 1 hr. Using a vortex mixer, 4 mL of toluene was added and blended for 20 sec. After that, a glass test tube was gently pipetted into the container for the toluene phase and the absorbance was measured at 520 nm using a UV-Visible spectrophotometer. A proline standard curve was used to calculate the prolines' amount, which was reported as µg/g FW.

Statistical analysis

The effects of UV-B+, water stress and a combination of stress were assessed using one-way ANOVA in SPSS 17.0 and the differences were declared statistically significant at $p < 0.01$. For each set of test settings, three duplicate assays were used.

Results

After 5 days of growth in well water, *R. sativus* seedlings were subjected to water stress (substitute days), long-term UV-B radiation (4 hr/day) and a combination of water and UV-B stress. Following exposure to stress, samples were taken at regular intervals to examine several differences such as development, non -photosynthetic and photosynthetic pigment synthesis, antioxidant activity and proline aggregation. Tropical areas have ambient UV-B levels that are at least 60 % greater than those in temperate areas. Under tropical conditions, *R. sativus* with subsurface storage organs grew well. More UV-B radiation and water stress exposure led to better changes in the plant growth characteristics.

Growth

Radish seedling growth traits have been frequently linked to stress (Fig. 1). According to the findings, plants' reactions to many stressors were noticeably different from those under single-stress settings. The combined stress treatment had a more notable effect on the key growth indices of *R. sativus* seedlings than UV-B irradiation and water stress. Water stress and UV-B irradiation together reduce growth factors, including fresh, dried weight and Plant height (Fig. 2). Both the leaf area and plant height were significantly impacted by the combined stress. The combined impacts of UV radiation and water stress were much more powerful than either stress component acting on its own.

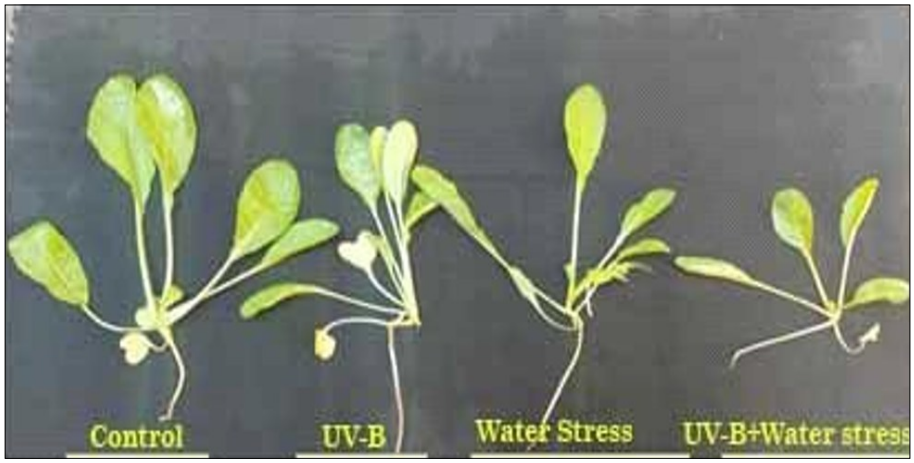

Fig. 1. Effects of well-irrigated, UV-B+, water stress and combinations of stress on *R. sativus* growth parameters (UV-B + water stress).
The results are the average of three different measurements (n=3)

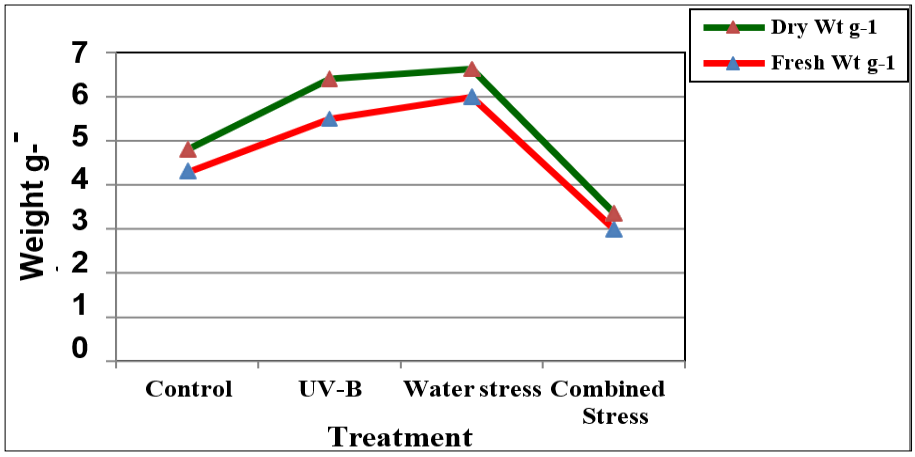

Fig. 2. Change in *R. sativus* fresh and dry weight when grown in well-irrigated, UV-B+, water stress and combined stress (UV-B + water stress).
The results are the average of three different measurements (n=3)

Photosynthetic pigment variation

Various times were used to examine the alterations in photosynthetic pigments in plants exposed to ambient, UV-B, water stress and combination stress (UV-B + water stress). Both pressures had varied responses in relation to chlorophyll content. Chlorophyll content, overall chlorophyll levels and statistically insignificant relationships with ambient chlorophyll levels all fell as a result of the combined operation of both stresses (Fig. 3, Table 1). Each of these stresses increased the production of chlorophyll. But it was more obvious in plants that had received UV-B treatment.

Table 1. Chlorophylls measurement

Chlorophyll a (mg/L)	$(12.21 \times A_{663}) - (2.81 \times A_{646})$
Chlorophyll b (mg/L)	$(20.13 \times A_{646}) - (5.03 \times A_{663})$
Total chlorophyll (mg/L)	$(7.18 \times A_{663} + 17.32 \times A_{646})$

Carotenoids

To ensure the removal of the chlorophyll and membrane, carotenoids function as light-harvesting pigments that quench triplet chlorophyll and release oxygen from the excited chlorophyll-

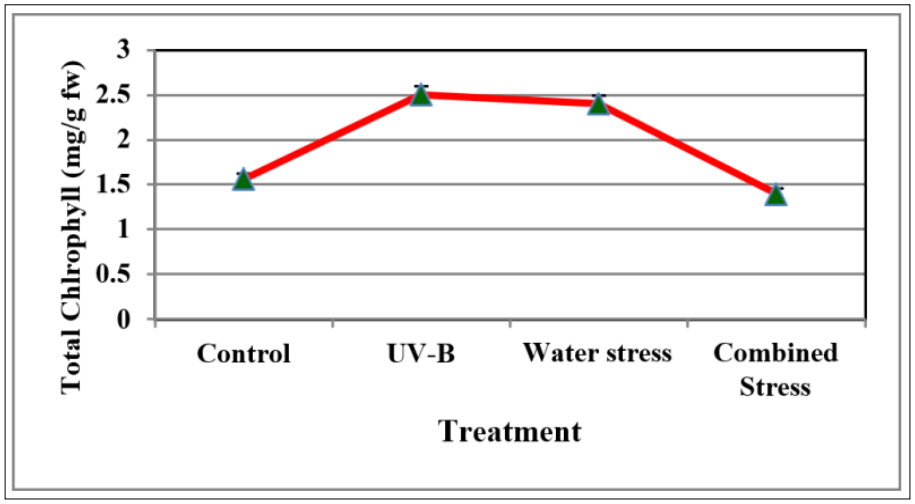

Fig. 3. Change in the amount of chlorophyll of *R. sativus* grown under well-irrigated, UV-B+, water stress and combination of stress (UV-B + water stress). The results are the average of three different measurements (n=3)

oxygen complex. When compared to ambient plants, the carotenoid level under stress remained stable. The concentration of carotenoids was not significantly affected by water stress or the two together, although they did affect carotenoid production in different ways. The combined stress boosted carotenoid production in addition to chlorophyll breakdown. When comparing the control plant, we found that the carotenoid production was higher in stress was given together and stress was applied alone. The carotenoid production was 50 % higher (Fig. 4).

Changes in the secondary pigments

In contrast to leaves grown under ambient solar radiation, leaves generated under conditions of increased water stress, UV-B radiation and combination stress (UV-B + water stress) have larger amounts of flavonoids, a non-photosynthetic UV-B absorbing pigment (Fig. 5). Although the amount of accretion varied depending on the stress environment, seedlings developing under combined stress conditions exhibited high flavonoids content (10 %).

The application of stress on the *R. sativus* seedlings did not affect the growth of anthocyanins (Fig. 6), but there was a considerable rise when water stress and UV-B were evaluated simultaneously, which was more pronounced after the use of UV-B light. Plants cultivated with UV-B enhanced radiation had a radish level that was more than 40 % higher than plants grown with ambient light.

Proline

In a stress-dependent way, varied stress conditions greatly elevated proline levels in *R. sativus* leaves. The response to water stress revealed a significant amount of proline content in *R. sativus* plants. Under underwater stress, the proline level increased noticeably and doubled over ambient and UV-B treated plants to 15.2 mmol/g FW, a significant increase. It appears that the combined stress state caused a substantially larger buildup of free proline (Fig. 7). However, in comparison to ambient plant, UV-B light significantly but marginally enhanced proline accretion.

Antioxidant activity

The majority of physiological stresses cause an oxidative burst by increasing the quantity of ROS, which in turn disrupts plant metabolism. On the other hand, there is a link between plant stress resistance and antioxidant capacity and higher antioxidant fraction levels can protect plants from stress (Fig. 8, 9).

In stressed radish plants, DPPH and hydrogen peroxide activity increased. The activity of DPPH was raised by ultraviolet-B irradiation, but not as much as peroxide activity. A decrease in DPPH and hydrogen peroxide activity was seen in the control plant. Although peroxidase activity was decreased in the control plant when compared to the stress condition, the difference was statistically insignificant. Significant changes in enzyme activity emerge from the combined operation of both stress factors,

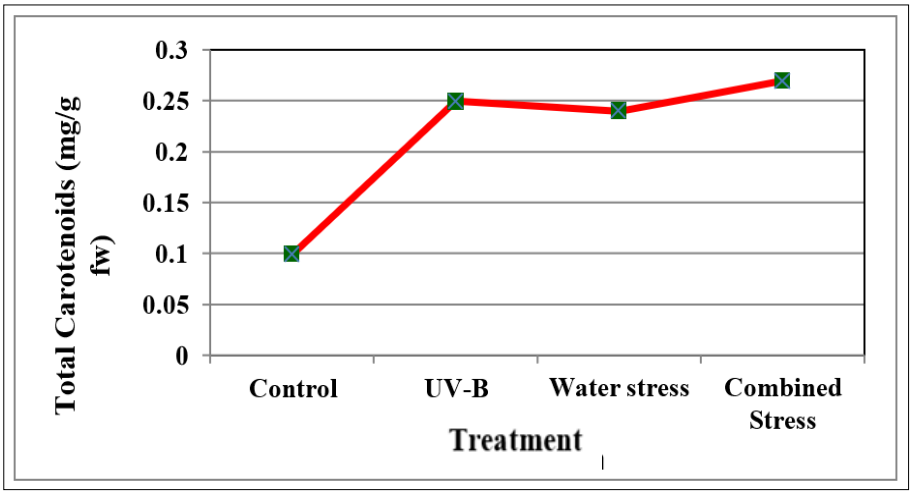

Fig. 4. Change in carotenoid content of *R. sativus* grown under well-irrigated, UV-B+, water stress and combination of stress (UV-B + water stress). The results are the average of three different measurements (n=3, Mean ± SE)

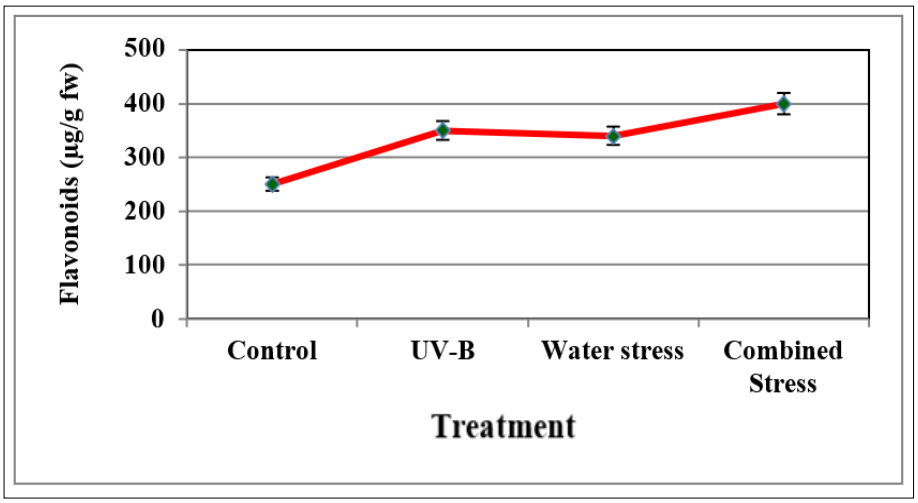

Fig. 5. Changes in the amount of flavonoids in *R. sativus* plants cultivated under water stress, UV-B+ and combinations of stress (UV-B + water stress). The results are the average of three different measurements (n=3, Mean ± SE)

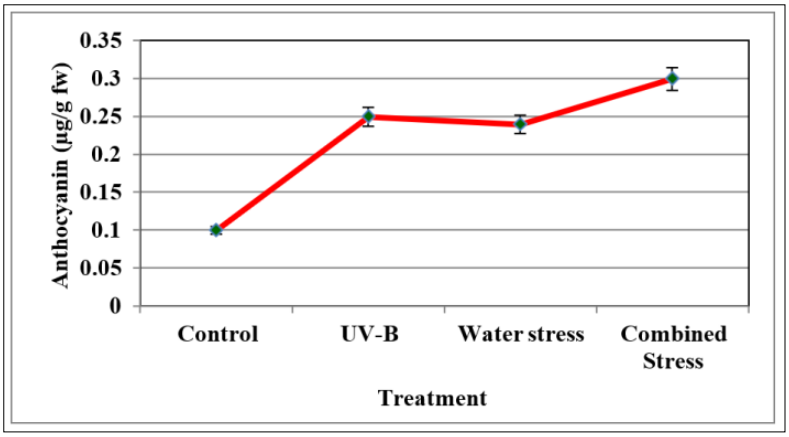

Fig. 6. Changes in the anthocyanin content of *R. sativus* grown under well-irrigated conditions, UV-B+, water stress and the combination of these conditions. The results are the average of three different measurements (n=3, Mean ± SE)

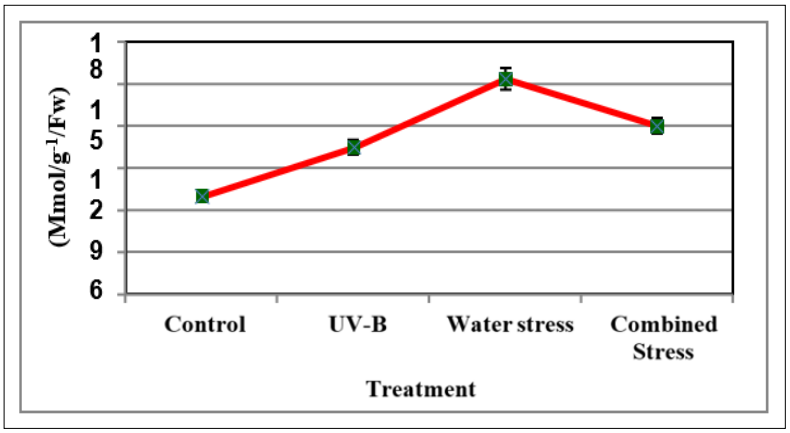

Fig. 7. Changes in the amount of proline content of *R. sativus* plants cultivated in well-irrigated, UV-B+, water-stressed and combined (UV-B + water-stressed). The results are the average of three different measurements (n=3, Mean ± SE)

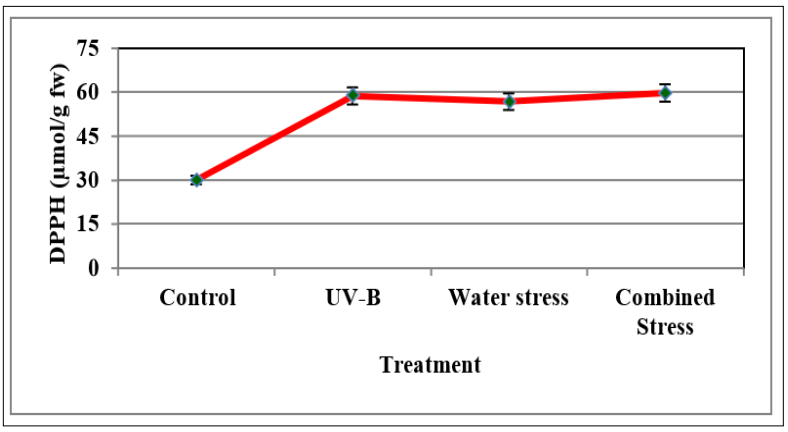

Fig. 8. Changes in the amount of DPPH produced by *R. sativus* plants cultivated in well-irrigated, UV-B+, water-stressed and combined (UV-B + water-stressed). The results are the average of three different measurements (n=3, Mean ± SE)

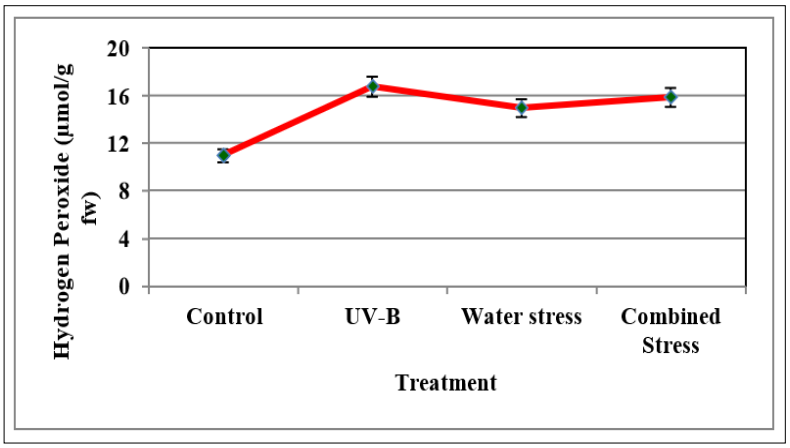

Fig. 9. Changes in the amount of hydrogen peroxide produced by *R. sativus* plants cultivated in well-irrigated, UV-B+, water-stressed and combined (UV-B + water-stressed) environments. The results are the average of three different measurements (n=3, Mean ± SE)

compared to the UV-B and drought functions acting alone. The findings demonstrated that when UV-B stress and drought stress were combined, UV-B stress performed better in terms of enzyme activity than drought stress.

Discussion

Although a link between UV-B exposure and the stress brought on by drought in plants has recently been demonstrated, the mechanism of vulnerability or resistance to combined stress has received less attention and is still unclear. The current study is an essential management approach for preventing UV-B and drought effects, which mostly affect field crops and focuses on some of the consequences and processes of UV-B and drought tolerance in higher plants. To analyse the numerous parameters, the plants were newly picked at the proper time. Plant growth, fresh weight and dry weight were all reduced as a result of the combined stress (UV-B and water stress) treatment; it outperformed the stress components' individual functions in strength. UV-B or drought. Under water stress, *R. sativus* seedlings were less vulnerable to UV-B than cucumber seedlings (15).

A substantial environmental stress known as drought has a detrimental effect on plant growth, slowing down stomatal activity, stem elongation, leaf expansion and growth rate (16). Research indicates that sunflower plants were found to benefit from being grown without UV-B radiation before being subjected to drought and further UV-B radiation (17). At the same time had no substantial impact on the amount of dry matter they produced or mitigated the effects of either element alone. Research has demonstrated that the morphology, biomass and physiology of *Verbascum thapsus* L. were most negatively impacted by the drought stress (18). However, only the shape and physiological features of leaves were significantly impacted by UV-B radiation. There is no proof that UV-B and drought have cumulative effects, nor are there any origin-dependent stress reactions, which would indicate that native or immigrant populations can adapt to their local environment (18). Similar effects were seen in maize plants grown outdoors without affecting the normal PAR to UV-B ratio, when UV-B radiation was assessed separately and shortly after the plant emerged. They have been demonstrated to be crucial for reducing the harm caused by UV-B to terrestrial plants (19). Additionally, research has demonstrated that both genotypic changes and assimilate usage changed the relationship between UV-B and water stress in soybean (20). Drought, UV-B exposure and the resulting stressors all significantly slowed the growth of wheat seedlings (measured by their fresh weight) (21). There is a lot of proof that dryness and UV-B damage wheat and pea growth. (Alexieva), soybean (Shen) and barley (Bandurska).

The effects of water stress or UV-B alone on plants drive the development of photosynthetic pigments; however, plants respond differently when both water stress and UV-B are present at the same time. During drought stress, numerous species have shown decreased chlorophyll and increased carotenoid levels. Depending on the pressures' duration and intensity (22). Increasing UV-B radiation caused some plants to produce more chlorophyll in outdoor conditions (23). This experiment also demonstrated that water stress had a major impact on chlorophyll content, while UV-B radiation had little to no impact. These results are believed to be in opposition to the majority of UV-B research, which are shown to have a detrimental impact on the chlorophyll in plants growing in growth chambers and outdoors and accelerated leaf withering in seedlings, just the water stress brought on by intense UV-B radiation leading to the depletion of photosynthetic pigments was the result of the considerable interaction effect on Chl a and b, which may have been brought on by a decrease in synthesis or an increase in degradation (24, 25). In contrast to water stress, UV-B radiation decreased the amount of chlorophyll in the environment, which was lower than the control level under both stresses (26). However, research has hypothesised that modifications in the growth of the photosynthetic pigment pool and activation of antioxidant enzymes may have acted as barriers to PSII irreversible phototoxicity following high UV-B exposure (27). Research from the past has described experiments where plants exposed to a single stressor have shown increased resistance to forthcoming hazardous environmental conditions. In rice subjected to LiCl and PEG, seedlings of cucumbers that have been heated and salted and grapes subjected to UV-B exposure and drought, cross-adaptation has been noted (28–30).

When compared to controls, carotenoid concentration rose under a single function of UV-B or drought conditions. When there was a discernible relationship between water stress and UV-B, it led to the production of more carotenoids than in the control. When exposed to soil moisture stress. Numerous crop species have been found to contain more carotenes and less chlorophyll (31). Despite being a crucial part of the plants' antioxidant defence system, carotenoids are susceptible to oxidation. Carotenoid concentration was significantly reduced in *Pisum sativum* and *Dolichos lablab*. Numerous defence mechanisms have been developed by various plants to assist them in surviving under temperature stress and poor light situations (32). Carotenoids are produced as the essential pigment for light harvesting. Carotenoid reduces ROS that are formed when vegetation is subjected to UV-B radiation, protecting chlorophyll components from photooxidation (16).

The primary pigment required for light harvesting is the production of carotenoids. Carotenoids protect the chlorophyll content from photooxidation by eliminating ROS that are produced when exposed to UV-B radiation (33). When stress is present, the radish plant produces 50 % more flavonoids and anthocyanins than under normal conditions. In most cases, the formation of secondary metabolites is coordinated with the preservation of plant tissue against stress. An increase in ultraviolet -B radiation has a major impact on the pathways for flavonoids and changes the flavonoid profiles of various plants (34, 35). The UV-B dosage modifies the impact of ultraviolet light, structure of flavonoids and other environmental factors, including temperature, light quality and intensity and the density of photosynthetic photons (7, 21, 36). UV-B exposures' intensity, duration and wavelength have an impact on the accumulation of flavonoids (37). Higher UV-B radiation levels typically promote the accumulation of flavonoids in plants. On the synthesis of secondary metabolites, such as flavonoids and anthocyanins, dryness and UV-B interaction had a substantial impact. Secondary metabolites accumulated greater under the combined stress than under control. When pea plants were exposed to dryness, prior treatment with a high UV-B to PAR ratio boosted flavonoid synthesis (38). In contrast, drought stress and greater UV-B

exposure significantly decreased the induction of anthocyanins and flavonols in barley by UV-B and pea (39, 40). Similar findings have been made regarding the cultivation of Silver Birch (*Betula pendula*) plants, which show increased flavonoid production in both young and older leaves when exposed to ambient PAR and UV-B radiation (41). Anthocyanin and flavonoids have been discovered to accumulate in wheat under drought stress, in peas as soluble phenols, as well (*P. sativum*) (26, 42).

To shield its cellular machinery from external stress, a plant develops a large number of small organic compounds known as osmolytes (43). The only stress marker that changed solely in response to drought stress, in either pea or wheat seedlings, was proline. Reactive oxygen species, which can enter through cell membranes and damage cells, are known to be produced as a result of dryness. This is supported by the discovery of proline in drought-stressed plants (44). Similar increases in the concentration of stress markers were seen in wheat and tobacco plants under drought stress (1, 45). In both water stress and combination stress conditions, the amount of such free proline amino acid was much higher. However, when compared to the ambient plant, UV-B radiation modestly increased free proline content in *R. sativus*. The osmolytes responsible for osmoprotection, free proline, may contribute to osmotolerance to various stresses. One of the universal characteristics of all abiotic stress is treated by the management and accumulation of osmoprotectant in the cell sap, a conserved aspect recognised in all plants, including those resistant and prone to stress (46). In either pea or wheat seedlings, only the stress marker proline increased in response to drought stress. When compared to control plants, several treatments significantly increased the DPPH radical scavenging capacity of radish seedlings. The combined effects of UV-B and drought, as well as the single function of stress components, were seen after 30 days of stress treatments, boosting DPPH radical scavenging activity by 30 %, with a definite effect. Increased amounts of antioxidant components may shield plants from stress damage, as antioxidant capacity has been associated with plant stress resistance. The interaction effects of UV-B and drought could explain the increased scavenging capability of antioxidants generated by both stress conditions. An imbalance between the production and removal of free radicals in the photosynthetic and respiratory pathways has been linked to stress-related oxidative damage. Research has discovered that the degree of plant seedling stress tolerance was correlated with a greater rise in both ABTS and DPPH radical scavenging abilities (47, 48). In contrast to drought-sensitive plants, drought-tolerant plants often had a large antioxidant system capacity that rose many times above Unstressed plants' reactions to stressful circumstances (49).

The present investigation shows that both stress situations result in an increase in the hydrogen peroxide level of *R. sativus* seedlings. However, in the combined stress-treated plant, the increase was more noticeable. The amount of active oxygen produced and utilised by enzyme systems is gauged by the presence of hydrogen peroxide. Changes in SOD activity and overproduction of H_2O_2 in sunflower cotyledons and *Picea asperata* seedlings as a result of exposure to UV-B radiation have been documented (50, 51). Research has demonstrated that plants exposed to high amounts of UV-B radiation had a more pronounced antioxidant enzyme activity (28). Furthermore, when plants were subjected to either water scarcity or a combination of both conditions, hydrogen peroxide production increased dramatically. Drought and heat stress significantly increased hydrogen peroxide accumulation, causing plants exposed to both conditions to accumulate more, which had a significant impact on leaf lipid peroxidation (MDA accumulation), Electrolyte Leakage (EL) and membrane stability index. The activity of the antioxidant system enzymes CAT, GPX and SPX, which catalyse hydrogen peroxide elimination processes, increased in cucumber cotyledons as H_2O_2 accumulated. In tomato plants, UV-B irradiation boosted the catalase activity (52). Several plants, including barley, sugar beet and sugar cane, have shown increased peroxidase activity when exposed to UV-B radiation (53).

Conclusion

Raphanus sativus plants responded more adversely to combined UV-B and water stress than to individual stresses, showing reduced growth, biomass and dry matter. Combined stress decreased chlorophyll content, though overall levels remained relatively stable, while non-photosynthetic pigments were more sensitive to the combined stress. Stress-induced fluctuations followed a similar trend across treatments, with water deficiency driving an increase in free proline under both water and combined stress. Antioxidant accumulation and enzyme activities were significantly altered under all stress conditions, with both individual and combined treatments contributing to these changes. Although UV-B alone promoted growth by mitigating some stress effects, further research is needed on the roles of stress duration, exposure timing and plant-specific traits in stress adaptation.

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Authors' contributions

NS designed the framework, provided guidance and validation and reviewed and edited the manuscript. PJPSM assisted in designing experiments, developing protocols, conducting fieldwork and collecting data. MR designed experiments and protocols, conducted fieldwork, collected data and performed validation. MS critically reviewed and revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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