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Research Article

# Early Growth Responses Towards UV-B, Drought and their Interaction in Mungbean

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## Abstract

Ultraviolet (UV) solar radiations have received greater attention during recent years, mainly due to depletion of stratospheric ozone and hence their consequent increase. The heightened incidence of drought stress resulting from climate change is creating a challenging environment for plant life. The aim of the present study was to investigate the response of mungbean seedlings to these stressors singly and in combinations (UV-B and PEG 6000) on seed germination and early seedling growth parameters. Mungbean (*Vigna radiata* L.) is a leguminous species grown in different parts of the world, especially in Asia including India where it is a common source of protein in the nutrient supplements. The seeds were exposed to 2 h of UV-B irradiation for three days and drought condition was generated by supplying polyethylene glycol 6000(PEG 6000) solution of 10% for five days. The combined stress was given by supplying UV-B (2h for 3 days) + 10% PEG (for 5 days) together. After five days, early growth parameters, enzymes related to seed germination (total amylase and protease) and biochemical parameters were analyzed in seedlings. The results revealed that both UV-B and drought stress have substantial negative effects on percent germination, root, shoot and seedling length, fresh and dry weight of seedlings, vigour index-I and II. UV-B and drought exposure independently caused significant inhibition of total amylase and protease activities in roots and shoots of 5 day old mungbean seedlings. Further, the oxidative stress-associated markers such as proline and hydrogen peroxide ($H_2O_2$) were found to increase in response to both the stresses in root as well as shoot tissues. Exposure to both UV-B radiation and drought stress negatively affect various indicators of plant growth and health, including increased production of $H_2O_2$, which can damage crucial biomolecules like DNA, RNA, and proteins. The observed reductions in germination, seedling growth, vigor, and key biochemical parameters suggest a synergistic interaction between these two stressors. Despite these negative impacts, simultaneous increase in proline and $H_2O_2$ levels under UV-B, PEG (drought), and combined stress conditions may point towards an adaptive response and a degree of tolerance to these stressors.

**Keywords:** Drought; Growth; Oxidative Stress; UV-B; Tolerance

## Introduction

Plants are exposed to various abiotic environmental stress factors that influence their growth and development throughout their life cycle. Plants are more vulnerable to heatwaves, floods, droughts, rising temperatures, salinity and Ultraviolet radiation (UV). In the universe, solar radiation is the major source of energy and critical for the plants growth and development. UV radiation is a part of the spectrum of electromagnetic rays emitted by the sun. It is divided into three bands of different wavelengths that are UV-A (400-320 nm), UV-B (320-290 nm), and UV-C (290-200 nm). UV-C is totally absorbed by stratospheric ozone layer, and it has minimal penetration to the Earth surface; by 90% or more of UV-B is also absorbed by stratospheric ozone layer, while UV-A passes through the atmosphere [1]. Despite the implementation of the Montreal Protocol, the long atmospheric lifetime of chlorofluorocarbons (40-150 years) will likely prevent a full recovery of global

UV-B radiation levels to pre-industrialization levels by 2050 [2]. More UV-B radiation is reaching at the Earth's surface as a result of atmospheric pollution-induced stratospheric ozone depletion by chlorofluorocarbons [1,3]. Numerous studies demonstrated that enhanced UV-B radiation has considerable impact on the morphological, physiological, and biochemical processes of a wide range of plant species [1,4]. Even a small increase in incident UV-B radiation can have significant biological effects since UV-B is readily absorbed by a number of important macromolecules such as nucleic acids, proteins, lipids and phytohormones [1]. UV-B acts as a harmful agent causing damage to biomolecules by producing reactive oxygen species (ROS), which can cause oxidation of lipid and protein and damage to DNA [1]. Plants develop a wide range of defensive mechanisms to combat UV-B radiation. These mechanisms consist of DNA repair, the production of UV-B absorbing compounds such as carotenoids, flavonoids, and anthocyanins and leaf thickening [1,3,5]. However, the ability of these mechanisms to tolerate UV-B radiation differs amongst crop species [6].

Water deficit stress is a significant abiotic component that contributes to dehydration and osmotic imbalance in cells. As a result, the water potential of plant tissues is reduced, which has an impact on agricultural yields [7]. Since polyethylene glycol with a molecular weight range of 6000 or higher cannot pass through plant cell pores, PEG molecules have been widely used to induce water shortage in plants [8]. It also reduces seed germination percentage, root length, seedling water content, decreases chlorophyll content, and inhibits enzymes of chlorophyll biosynthesis and increases the activity of antioxidant enzymes [9,10].

Plants are continuously exposed to combinations of abiotic and biotic stressors. While much is known about responses to individual stressors, understanding of plant responses to combinations of stressors is limited. Drought, a prevalent environmental stressor, frequently coincides with high UV radiation, significantly impacting agricultural productivity and product quality [11]. Global attention has become focused on increasing UV-B intensity and drought (due to low rainfall), which can have negative impacts on ecological and biological systems [12,13]. It has been found that the combined effect of UV-B radiation and drought stress leads to alterations and reductions in growth, physiological and biochemical processes in plants [13,14]. The studies on combined effects of UV-B and drought stress in plants, including mungbean are not available. Mungbean is a versatile legume, consumed both as whole grains and sprouts. It is a rich source of protein and also contributes to sustainable agriculture by enriching the soil through nitrogen fixation [15]. Hence, the present study is aimed to analyze the effects of UV-B radiation and drought (induced by PEG 6000) and their combination on seed germination and early growth characteristics, biochemical parameters (DNA, RNA and Protein) and oxidative stress marker ($H_2O_2$ and proline) in mungbean seedlings with a view to elucidate the mechanistic details.

## Materials and Methods

### Material and growth conditions

The **s**eeds of local variety of mungbean (*Vigna radiata* L.) were obtained from the market of Indore (M.P.), India. Preliminary experiments were performed and the experiments suggested that UV-B irradiation 2 hours for three days was adequate time of UV-B stress to mungbean seedlings. UV-B irradiation was applied artificially through UV-B fluorescent lamps (20 W, γ max 315 nm, Philips, the Netherlands). These lamps were positioned 45 cm above the Petri plates to deliver the desired UV-B irradiation. The intensity of UV-B radiation was 2.9 mW $cm^{-2}sec^{-1}$ measured by radiometer, Solar light Co. Inc. (PMA 2100), Glenside, PA, USA. The drought conditions were generated by supplying polyethylene glycol 6000 solutions of 10% for 5 days. Four treatments were applied to mungbean seeds to investigate stress responses: (1) Control: Seeds were grown under normal conditions, receiving no UV-B radiation and being well-watered with distilled water. (2) UV-B Treatment: Seeds were exposed to UV-B irradiation for 2 hours per day for 3 consecutive days. (3) Drought Treatment: Drought stress was induced by supplying 10% polyethylene glycol 6000 (PEG 6000) solution to seeds for 5 days. (4) Combined Stress Treatment: Seeds were subjected to both UV-B irradiation (2 hours/day for 3 days) and drought stress (10% PEG 6000 for 5 days) concurrently.

### Germination and growth parameters

### Germination percentage

Uniform and healthy seeds of mungbean (*Vigna radiata*) were selected and surface-sterilized by soaking in 0.01% $HgCl_2$ solution

for 1-2 min and then washed thoroughly with tap water followed by distilled water. Seeds were placed in 15 cm diameter Petriplates (10 seeds per plate) lined with moist Whatman no.1 filter paper discs with 10 ml of distilled water/PEG solution and then seeds were exposed to UV-B. After 72 hours of seed imbibition, the percentage germination was calculated by counting the number of germinated seeds relative to the total number of seeds by using formula of Close and Wilson [16].

### Growth Parameters

After five days of growth in complete darkness, seedling, root, and shoot lengths were measured. Measurements were taken using thread and a centimeter scale, with seedling length measured from root tip to shoot tip, and root and shoot lengths measured separately from their junction to their respective tips.

### Vigor Index I and II

Five-day-old seedlings were selected from each replicate in order to measure the fresh and dry weight of the root, shoot and seedlings. After drying in an oven for 3 days at 80 °C, the root, shoot and seedlings were weighed collectively to determine their consistent dry weight (Electrical weighing balance was used). The following formula, provided by Abdul-Baki ans Anderson [17], was used to determine the vigor of seedlings Vigor index I = Germination % × Seedling length

- Vigor index II = Germination % × Seedling dry weight

### Total amylase activity

For the total amylase enzyme activity, 100 mg of germinated mungbean root and shoot tissues were crushed using a mortar and pestle in 5.0 ml of chilled 80% acetone and centrifuged for 10 min at 4 °C at 10,000 rpm. The supernatant was disposed of, and the pellet was taken again and mixed with 10.0 ml of 0.02 M phosphate buffer (pH 6.4). This mixture was then centrifuged at 12,000 rpm for 15 minutes at 4°C yielding supernatant was utilized as an amylase enzyme source. Total-amylase activity was calculated using the Sawhney., *et al*. [18]. The amount of starch hydrolyzed $h^{-1}$ $g^{-1}$ fresh weight of seedlings was reported as the total amylase enzyme activity.

### Total protease activity

Root and shoot tissues from germinated mungbean seedlings were homogenized in 5.0 ml of phosphate buffer (0.2 M, pH 7.6) using a cold mortar and pestle. After passing through Whatman No. 1 filter paper, the homogenate was centrifuged for 30 minutes at 4 °C at 12,000 rpm. By using Kunitz's method [19] for the enzyme assay, supernatant was utilized. The amount of mg protein hydrolyzed $g^{-1}$ fresh weight of seedlings represented as protease enzyme activity.

### Total Proline Content

The method of Bates., *et al*. [20] was used to quantify the proline content. Using a mortar and pestle, 250 mg of root and shoot tissues were homogenized in 5.0 ml of 3% (w/v) aqueous sulphosalicylic acid; the homogenate was centrifuged for 10 minutes at 10,000 rpm. The mixture of 2.0 ml acid ninhydrin reagent and 2.0 ml glacial acetic acid was added to 0.5 ml of supernatant. The mixture was kept for one hour boiling at 100 °C. After cooling in an ice bath, 4.0 ml of toluene was added to stop the reaction. Following complete mixing, the toluene-containing chromophore was isolated, and the red color generated absorbance was measured at 520 nm and expressed as μg $g^{-1}$ fresh weight of seedlings. The standard curve (5-25 μg proline) was used to calculate proline content.

### Hydrogen peroxide ($H_2O_2$) content

$H_2O_2$ was estimated using the titanium hydroperoxide complex synthesis method of Mukherjee and Choudhari [21]. Cold acetone (5.0 ml) was used to grind 0.5 g of mungbean root and shoot tissues into a paste using a cold mortar and pestle. After the homogenate was sieved through Whatman No. 1 filter paper, 2.5 ml of ammonium hydroxide solution and 2 ml of titanium reagent were added to precipitate the $H_2O_2$-titanium complex. The reaction mixture was centrifuged at 10,000 rpm for 5 minutes at 4°C. After dissolving in 2.0 ml of 2 M concentrated sulfuric acid ($H_2SO_4$), the precipitate was centrifuged again. The intensity of yellow color of the supernatant was measure at 415 nm using a double beam spectrophotometer (Shimadzu UV -1800). The $H_2O_2$ content was calculated by the standard curve prepared in the range 20-200 μmole $H_2O_2$ and expressed as μmole $H_2O_2$ $g^{-1}$ fresh weight.

### Total DNA content

Root and shoot tissues (1g) were boiled in 5.0 ml of 80% ethanol for 3 min. The tissue was then homogenized in 5.0 ml of 80% ethanol and centrifuged. After discarding supernatant, the pellet obtained was mixed with 2.0 ml of 1% PCA and centrifuged. The pellet was then treated with 5.0 ml of ethyl alcohol: diethyl ether: chloroform (2:2:1) mixture. After centrifugation, the supernatant was discarded and to the resulting residue, 3.0 ml of 0.3 N KOH was added. The mixture was kept for about 18 h at 37°C. After incubation, the medium was adjusted to pH 2.0 by adding IN PCA. The mixture was centrifuged and pellet obtained was used for DNA estimation using the method of Gendimenico., *et al.* [22] by using diphenylamine (DPA) reagent. The DNA content was calculated through standard curve prepared with 40-400 µg of horse sperm DNA.

### Total RNA content

Total RNA content was measured using Orcinol reagent by the method given by Webb and Levy [23]. The root and shoot tissues (250mg) were boiled in 5.0 ml of 80% ethanol for 3 min. The tissue was then homogenized in 5.0 ml of 80% ethanol and centrifuged at 5000 rpm for 10min at room temperature. After discarding supernatant, the pellet obtained was mixed with 2.0 ml of 1% PCA and centrifuged. The pellet was treated with 5.0 ml of ethanol: diethyl ether: chloroform (2:2:1) mixture. After centrifugation, the supernatant was discarded and to the resulting residue, 3.0 ml of KOH (0.3 N) was added. The mixture was allowed to stand for about 18 h at 37°C. After incubation, the pH of the medium was adjusted to 2.0 by adding 1 N PCA. The contents were centrifuged and supernatant obtained was used for RNA estimation. The volume of the supernatant was made up to 5.0 ml with 1 N PCA. Diluted supernatant (0.2 ml) was mixed with 1.8 ml of 1 N PCA and 5.0 ml of freshly prepared Orcinol reagent. The mixture was boiled in a boiling water bath for 30 min and cooled. The absorbance of the green color formed was measured at 675 nm using a double beam spectrophotometer (Shimadzu UV -1800). The total RNA content was calculated using standard curve prepared in a range of 20-200 µg of yeast RNA.

### Total protein content

For the total protein estimation Lowry's method [24]. was used. The root and shoot samples (200 mg) were boiled for about 3 min in 5.0 ml of 80% ethanol. After that tissues were transferred and homogenized with 5.0 ml 80% ethanol, and the homogenate was centrifuged at 5000 rpm for 15 min. The obtained pellet was then suspended in 5.0 ml of 10% TCA, incubated for 30 min and centrifuged again at 12000 rpm for 12min. Supernatant was discarded and the pellet was dissolved in 5.0 ml NaOH (0.1N). The mixture was incubated for 20 min and centrifuged. The supernatant was then used to estimate total protein using Folin and Ciocalteu's phenol reagent. An aliquot of 0.5 ml of extract was mixed with 0.5 ml distilled water and 5.0 ml of Reagent C. After incubation for 15 min, 0.5 ml of Folin-Ciocalteu's Phenol was added. The mixture was allowed to stand for 30 min and the absorbance was taken at 660 nm using a double beam spectrophotometer (Shimadzu UV -1800). Protein content was determined using a standard curve (25-250 µg BSA).

### Statistical analysis

All the data presented in this study represent the mean ± standard error derived from three independent experimental replicates. Data analyzed by the Student's t-test $^{*}p < 0.05$,$^{**}p < 0.01$, $^{***}p < 0.001$ denote significant difference of roots; $^{\#\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ denote significant difference of shoots and $^{++}p < 0.05$,$^{++}p < 0.01$; $^{+++}p < 0.001$ denote significant difference of mungbean seedlings grown under unstressed (control) and different treatment conditions.

## Results

The germination percentage and early growth characteristics such as root length, shoot length, and seedling length, fresh weight and dry weight of root, shoot, and seedling, and vigour indices were reduced by UV-B radiation (2 hours), drought (PEG 10%), and the combined stress of UV-B and PEG (Table 1-3). Table 1 showed that, in comparison to the control, the germination percentage decreased by 50% in response to UV-B irradiation, 30% in response to drought, and 60% in response to combined stress.

Similarly, drought stress and UV-B radiation, both singly and in combination, greatly reduced the length of the roots, shoots, and seedlings (Table 1). UV-B and drought have been found greater impact than either one alone. This combined co-stress provoked remarkable deviations from the control; root length was reduced by 49%; shoot length and seedling length were decreased by 50% and 51% respectively (Table 1). The fresh weight and dry weight of root, shoot and seedlings were also significantly reduced by individual and combined stress of UV-B and drought (Table 2,3).

| Treatment | Germination Percentage % | Root length (cm) | Shoot length (cm) | Seedling length(cm) |
|---|---|---|---|---|
| Control | 100 ± 0.00 (100) | 4.806 ± 0.153 (100) | 3.963 ± 0.174 (100) | 9.118 ± 0.207 (100) |
| UV-B | 50 ± 3.086$^{***}$ (50) | 4.131 ± 0.244$^{**}$ (85) | 3.918 ± 0.278$^{ns}$ (99) | 8.049 ± 0.273$^{++}$ (88) |
| PEG | 70 ± 3.086$^{**}$ (70) | 4.412 ± 0.360$^{*}$ (91) | 3.462 ± 0.284$^{\#}$ (87) | 8.231 ± 0.581$^{+}$ (90) |
| UV-B + PEG | 40 ± 3.086$^{***}$ (40) | 2.462 ± 0.112$^{***}$ (51) | 2.012 ± 0.116$^{\#\#\#}$ (50) | 4.537 ± 0.198$^{+++}$ (49) |

**Table 1:** Individual and interactive effect of UV-B and drought stress on germinaton percentage root, shoot and seedling length in mungbean.

(In parentheses, the relative values in terms of changes between control germination percentage, and different treatments used are mentioned).

Value is mean ± SE and relative values are given in parentheses ns= non-significant, *= p < 0.05, ** = p < 0.01 and *** =p < 0.

| Treatment | Root Fresh weight (g) | Shoot Fresh weight (g) | Seedling Fresh weight (g) |
|---|---|---|---|
| Control | 0.067 ± 0.002 (100) | 0.135 ± 0.005 (100) | 0.271 ± 0.011 (100) |
| UV-B | 0.054 ± 0.002$^{**}$ (79) | 0.108 ± 0.005$^{\#\#}$ (80) | 0.216 ± 0.011$^{++}$ (79) |
| PEG | 0.064 ± 0.002$^{ns}$ (94) | 0.128 ± 0.005$^{ns}$ (94) | 0.256 ± 0.011$^{+}$ (94) |
| UV-B + PEG | 0.041 ± 0.003$^{**}$ (61) | 0.083 ± 0.006$^{\#\#\#}$ (61) | 0.167 ± 0.013$^{++}$ (62) |

**Table 2:** Individual and interactive effect of UV-B and drought on fresh weight of root, shoot and seedlings of mungbean.

(In parentheses, the relative values in terms of changes between control and different treatments used are mentioned).

Value is mean ± SE and relative values are given in parentheses ns= non-significant, *= p < 0.05, ** = p < 0.01 and *** =p < 0.001.

| Treatment | Root Dry weight (g) | Shoot Dry weight (g) | Seedling Dry weight (g) |
|---|---|---|---|
| Control | 0.027 ± 0.001 (100) | 0.015 ± 0.001 (100) | 0.042 ± 0.001 (100) |
| UV-B | 0.013 ± 0.001$^{***}$ (48) | 0.010 ± 0.002 $^{\#\#}$ (66) | 0.023 ± 0.003 $^{+++}$ (54) |
| PEG | 0.017 ± 0.001$^{**}$ (63) | 0.011 ± 0.001$^{\#\#}$ (73) | 0.035 ± 0.002 $^{++}$ (83) |
| UV-B + PEG | 0.012 ± 0.001$^{***}$ (44) | 0.011 ± 0.001$^{\#\#}$ (73) | 0.022 ± 0.003 $^{+++}$ (52) |

**Table 3:** Individual and interactive effect of UV-B and drought on dry weight of root,shoot and seedlings of mungbean.

(In parentheses, the relative values in terms of changes between control and different treatments used are mentioned).

Value means SE and relative values are given in parentheses ns= non-significant, * = p < 0.05, ** = p < 0.01 and *** =p < 0.001

The patterns of vigor indices I and II were comparable to those of seedling length and dry weight. The seed vigor index-I and II of mungbean seedlings significantly decreased by UV-B and drought separately as well as in response to combined stress (Table 4).

| Treatment | Vigour Index 1 | Vigour index II |
|---|---|---|
| Control | 911.87 ± 20 (100) | 4.2 ± 0.182 (100) |
| UV-B | 402.45 ± 13*** (44) | 1.15 ± 0.157*** (27) |
| PEG | 576.18 ± 14** (63) | 2.45 ± 0.160*** (58) |
| UV-B + PEG | 181.50 ± 10*** (19) | 0.88 ± 0.127**** (21) |

**Table 4:** Individual and interactive effect of UV-B and drought on vigour index I and II of mungbean.

(In parentheses, the relative values in terms of changes between control and different treatments used are mentioned).

Value are mean ± SE and relative values are given in parentheses ns= non-significant, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

In mungbean seedlings, total amylase activity was observed to decrease in response to combined stress as well as UV-B exposure and drought independently (Figure 1). In comparison to the control, there was a remarkable decline of 51% by UV-B and 19% by PEG was noted in amylase activity of roots (Figure 1). Additionally, when combined UV-B and drought stress was given then the amylase activity of the root tissues was significantly decreased by 65% as compared to control. A similar pattern of declining amylase activity was noted also in shoot tissue (Figure 1).

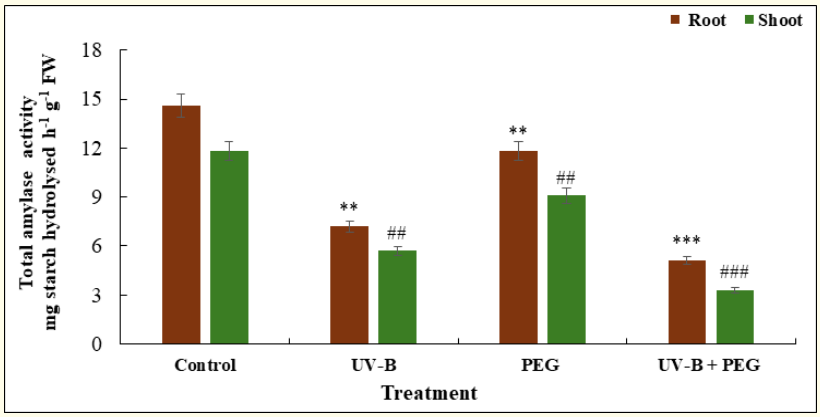


**Figure 1:** Individual and interactive effect of UV-B and drought stress on total amylase activity in mungbean seedlings. The vertical lines on bar indicates ± SE for mean and data analyzed by the Student's t-test **$p < 0.01$; ***$p < 0.001$ denote significant difference between roots under unstressed (control) and different treatments; ##$p < 0.01$; ###$p < 0.001$ denote significant difference between shoot under unstressed (control) and different treatment conditions.

The decrease in hydrolyzed protein per gram of fresh weight in the mungbean seedlings was attributed to decreased protease activity. The root and shoot tissue showed a considerable decrease in protease activity when exposed to UV-B and drought stress, both individually and in combination (Figure 2). The root tissues displayed a 40% reduction in protease activity in response to UV-B irradiation, and 20% reduction in response to drought, and 72% decrease in response to combined stresses. Identical pattern of decrease in protease activity was also seen in the shoot tissues of the mungbean seedlings (Figure 2); however the extent of reduction was more in roots in comparison to the shoot tissues.

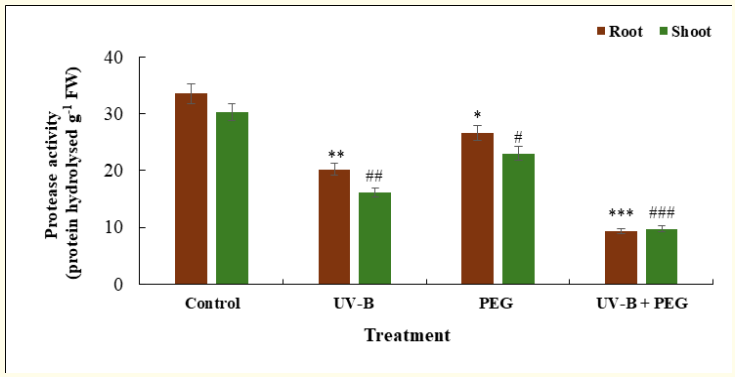


**Figure 2:** Individual and interactive effect of UV-B and drought stress on protease activity in mungbean seedlings. The vertical lines on bar indicates ± SE for mean and data analyzed by the Student's t-test, *p < 0.05; **p < 0.01; ***p < 0.001 denote significant difference between roots under unstressed (control) and different treatments; #p < 0.05; ##p < 0.01; ###p < 0.001 denote significant difference between shoot under unstressed (control) and different treatment conditions.

A tremendous increase in proline content was noticed when seedlings were exposed to UV-B and drought stress, both individually and in combination also (Figure 3). Indistinguishable pattern of increase in proline level was detected in the root tissues with several fold enhancements compared to the shoots (Figure 3). The shoot tissues showed a 98% increase in proline in response to UV-B irradiation, 83% increase in response to drought, and a synergistic increase in response to combined stresses (Figure 3).

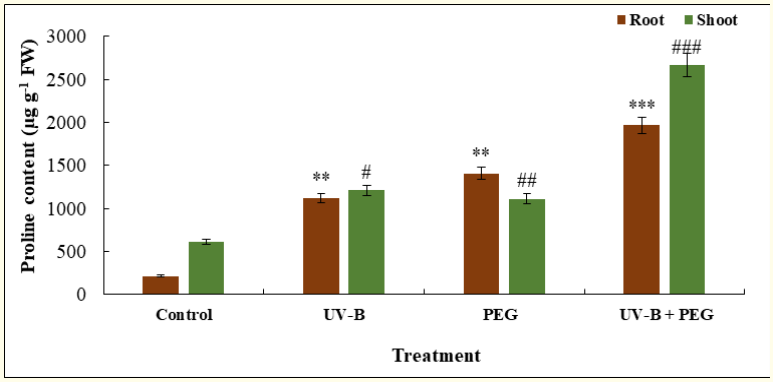


**Figure 3:** Individual and interactive effect of UV-B and drought stress on proline content in mungbean seedlings. (The vertical lines on bar indicates ± SE for mean and data analyzed by the Student's t-test, **p < 0.01; ***p < 0.001 denote significant difference between roots under unstressed (control) and different treatments; #p < 0.05; ##p < 0.01; ###p < 0.001 denote significant difference between shoot under unstressed (control) and different treatment conditions.

Significant increase in $H_2O_2$ content was observed after UV-B and drought stress, both individually and in combination also in root and shoot tissues (Figure 4). There was 147% increase in $H_2O_2$ content by UV-B exposure, 242% by PEG and 318% increase by the combined stress was noted in root tissues as compared to control (Figure 4). In shoot tissues there was 14% increase in $H_2O_2$ in response to UV-B irradiation, 98% increase due to drought, and a synergistic increase of 146% in response to combined stress (Figure 4).

The total DNA, RNA and protein content in root and shoot of mungbean seedlings was decreased in response to drought, and UV-B exposure independently and also by the presence of combined stresses (Figure 5-7). In comparison to the control, the notable decrease of 42% was observed in DNA content of roots by PEG. Additionally, when UV-B and drought conditions were induced simultaneously, the total DNA content decreased by 54%. A similar kind of declining pattern in total DNA content was found in shoot tissues of the mungbean seedlings (Figure 5).

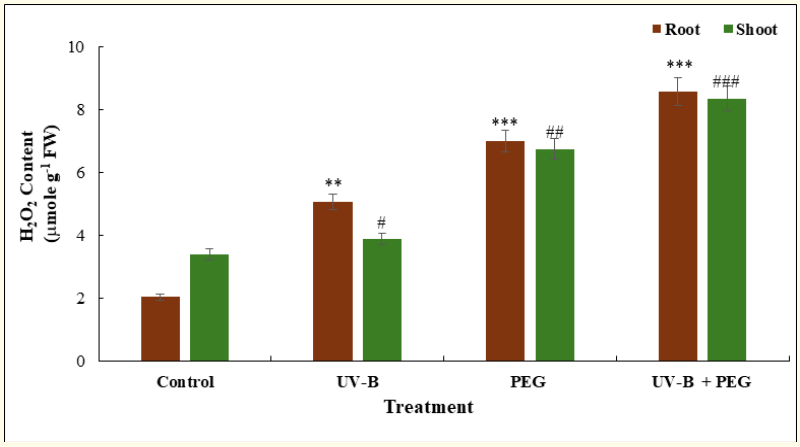


**Figure 4:** Individual and interactive effect of UV-B and drought stress on hydrogen peroxide content in mungbean seedlings. The vertical lines on bar indicates ± SE for mean and data analyzed by the Student's t-test, **p < 0.01; ***p < 0.001 denote significant difference between roots under unstressed (control) and different treatments; #p < 0.05; ##p < 0.01; ###p < 0.001 denote significant difference between shoots under unstressed (control) and different treatment conditions.

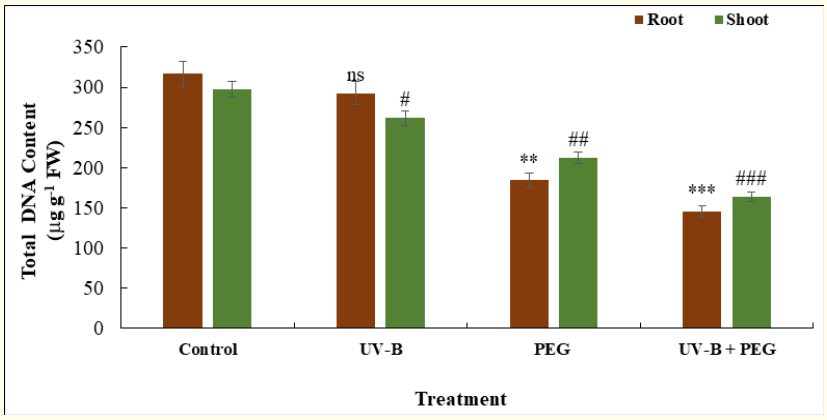


**Figure 5:** Individual and interactive effect of UV-B and drought stress on total DNA content in mungbean seedlings. The vertical lines on bar indicates ± SE for mean and data analyzed by the Student's t-test,**p < 0.01; ***p < 0.001 denote significant difference between roots under unstressed (control) and different treatments; #p < 0.05; ##p < 0.01; ###p < 0.001 denote significant difference between shoots under unstressed (control) and different treatment conditions.

RNA content in root tissues significantly decreased compared to the control; 16% by UVB exposure, 22% by PEG treatment, and 44% under combined UVB and drought stress (Fig.6). In shoot tissues, UVB irradiation led to a 19% decrease in RNA content, drought stress caused a 31% reduction, and the combined stresses resulted in a 47% decrease (Figure 6).

In comparison to the control, 26% decrease by UVB exposure, 13% was observed by PEG and 43% decrease when UV-B and drought conditions were given combined in total protein content of roots of mungbean seedlings (Figure 7). A comparable pattern of declining protein content was noted in shoot tissue of the mungbean seedlings (Figure 7).

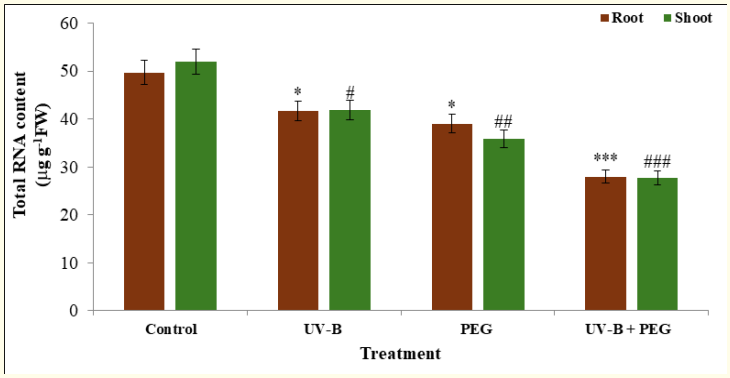


**Figure 6:** Individual and interactive effect of UV-B and drought stress on total RNA content in mungbean seedlings. The vertical lines on bar indicates ± SE for mean and data analyzed by the Student's t-test, *p < 0.05; **p < 0.01; ***p < 0.001 denote significant difference between roots under unstressed (control) and different treatments; #p < 0.05; ##p < 0.01; ###p < 0.001 denote significant difference between shoots under unstressed (control) and different treatment conditions.

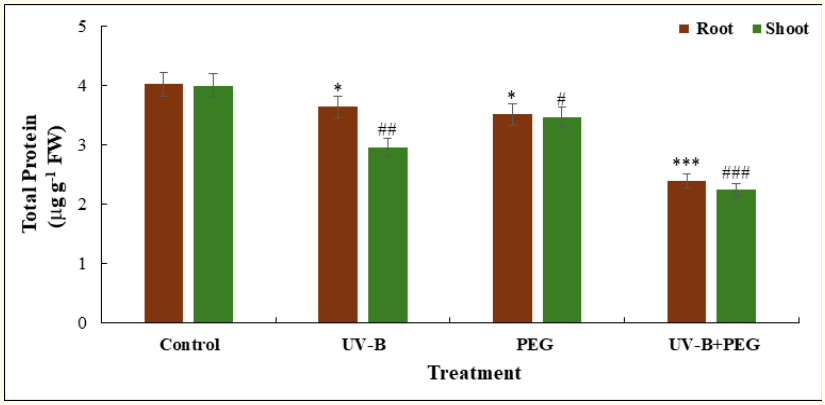


**Figure 7:** Individual and interactive effect of UV-B and drought stress on protein content in mungbean seedlings. The vertical lines on bar indicates ± SE for mean and data analyzed by the Student's t-test, *p < 0.05; ***p < 0.001 denote significant difference between roots under unstressed (control) and different treatments; #p < 0.05; ##p < 0.01; ###p < 0.001 denote significant difference between shoots under unstressed (control) and different treatment conditions.

## Discussion

Seed germination, essential for plant survival [25], is vulnerable to various abiotic stresses, including UV-B radiation, drought, high temperature, heavy metals, and salinity [26]. Plants exposed to UV-B radiations exhibit reduced physiological performance and morphological defects like plant stunting, lower seed germination, seedling growth, leaf area, biomass accumulation and productivity [27,28]. In the present study, growth performance of mungbean seeds was examined under the stress conditions produced by the exposure of UV-B radiation (2h for 3 days) and PEG 6000 10% (for 5 days). The results of present investigation revealed a significant decrease in percentage germination and early seedling growth parameters such as root, shoot, seedling length, fresh and dry weight and vigour index-I and II of mungbean seedlings by UV-B and drought stress singly (Table 1-4). The seedlings grown under combined stress conditions (UV-B + drought) exhibited substantial reduction in seed germination, biomass accumulation and vigour index-I and II than the seedlings grown under either only UV-B or only drought stress conditions (Table 1-4). Interaction between UV-B and drought stress was more than additive and they have synergistic effects on percentage germination, root, shoot and seedling length, and vigour indices as they were reduced respectively by 60, 49, 50, 51 and 80% as compared to control (Table 1-4). Alexieva., *et al*. [13] observed that both drought and UV-B radiation trigger an oxidative burst in pea plants. Choi., *et al*. [29] further demonstrated that PEG-induced drought stress inhibits cell division and elongation, resulting in shorter roots and shoots. This reduction in growth is likely linked to decreased cell wall elasticity, potentially caused by hormonal and hydraulic signals associated with drought conditions.

The significant reduction in root, shoot and seedling length by combined treatment of UV-B and drought stress in the present study indicates synergistic effect of both the stresses where PEG induced drought condition seems to be more severe in presence of UV-B. Thus, it appears that growing tissues Radicle (root) and plumule (shoot) are sensitive towards both the stressors. Reduction in root dry weight was more severe by UV-B radiation than the shoot indicating the sensitivity of roots to these radiations. Similar reduction in seedling growth on exposure to UV-B radiation has been reported for pine [30], soybean [31], tomato, radish and bean [32].

Researchers have frequently studied the effects of drought and high UV-B radiation on plants individually. However, studies investigating their combined impact are less common. Some studies have shown that drought and high UV-B radiation can have additive detrimental effects on plants, such as reduced plant height and leaf area in *Populus cathayana* [33] and decreased biomass in *Hippophae rhamnoide* [34]. In contrast, a study on soybeans found no evidence of additive effects of these two abiotic factors on growth, photosynthesis, and seed yield [35].

Seed germination process includes four steps which start with water imbibition, mobilization of food reserves, protein synthesis followed by radicle protrusion [36]. During germination α–amylase enzyme hydrolyses the stored endospermic starch into glucose and maltose which is to be utilized by growing embryo [37,38]. Proteases are involved in the hydrolysis of proteins in the germinating seeds [39]. Water stress decreases the activity of amylase enzymes, which has a detrimental effect on wheat seed germination and carbohydrate metabolism [40]. The results of present study also revealed that UV-B stress reduced the activities of total-amlyase and protease in roots and shoots of dark grown mungbean seedlings in control (UV-B) as well as after PEG treatment alone and in combination of these co-stressors (Figure 1,2). Similar results were obtained in soybean seedlings where UV-B reduced the total–amylase and protease activity and gene expression of amylase [28].

Under stress conditions, proline is recognized as organic solute in plants that act as an osmoprotectant, stabilize membranes and maintain water content [41,42]. The present investigation revealed an elevation in proline accumulation within root and shoot of mungbean seedlings when subjected to UV-B radiation, PEG as well as in their combinations (Figure 3). The marked increase in proline accumulation under UV-B in the present study is in agreement with the results of Balouchi., *et al*. [43] and this could represent adap-

tive response to oxidative damage induced by UV-B radiation. It has been suggested that UV radiation-induced proline accumulation protects plants against these radiation by scavenging the free radicals [41]. Elevated proline levels in plants subjected to PEG-induced water stress are also apparent to be an adaptive mechanism for stress tolerance [44]. It indicates with the general observation that plants cope with stresses by accumulating proline, which provides energy for growth and survival [41,42].

It is well suggested that reactive oxygen species such as hydrogen peroxide ($H_2O_2$) acts as a signaling molecule during the relief from dormancy and further progress towards germination. The substantial increase in ROS like $H_2O_2$, in root and shoot of mungbean seedlings was observed under all the stressors used in the present study (Figure 4). The significant effect of UV-B on the $H_2O_2$ content is in accordance with results of other researchers like Hassan., *et al*. [41]. and Prasad., *et al*. [45]. The contents of $H_2O_2$, and proline increased with PEG 6000 solution in mungbean [46].

In our results, we found reduction in DNA, RNA and protein content in root and shoot of mungbean seedlings in all the stress conditions. UV-B has the potency to cause protein degradation *via* destruction and/or modification of amino acid residues as well as through enhanced ROS production [47]. It has been found that altered DNA and RNA structures also hinder replication and transcription there by protein synthesis slow down under UV-B stress [48]. Protein content has been reported to decrease under UV-B radiation in pea [49] and mungbean [50] seedlings. Increased phenolics under elevated UV-B prevents this radiation for reaching the more photosensitive targets (such as the photosynthetic apparatus), act as defense compounds against UV-B induced oxidative stress, and Curtail UV-B induced damage to DNA [51]. Nucleic acids, particularly DNA, absorb strongly in UV-B region, so the in capitation of DNA can lead to visible damage in the plant. Consistent with previous findings, seedlings exposed to PEG-induced water stress in the present study exhibited a decrease in DNA, RNA, and protein levels (Figure 5,6,7). This reduction is primarily attributed to a down regulation of nucleic acid synthesis, a physiological response to reduced water availability and subsequent decline in metabolic activity under stress conditions [52]. RNA content and relative water content has been significantly reduced in leaves and roots with increased concentration of PEG in peanut [53]. To best of our knowledge this is the first report showing the reduction in total–amylase and protease enzyme activities, reduced DNA, RNA and protein content by the interaction of UV-B and drought stress in root and shoots of mungbean during early seedling growth.

## Conclusion

In conclusion, UV-B and drought stress, have detrimental effects in mungbean seedlings, which is apparent by the enhanced production of $H_2O_2$, which in turn adversely affect seed germination, total amylase and protease activities and health indicators DNA, RNA, and proteins. Interaction of these stressors could have additive or even synergistic effects which further impacting the mungbean roots and shoots drastically. Elevated levels of proline under both UV-B and drought stress singly as well as in their combinations advocate the potential of mungbean to withstand against UV-B, drought and their combinations. The results of present study suggest that mungbean seedlings are vulnerable to the combined effects of UV-B radiation and drought stress. This is a serious concern, as climate change is expected to lead an increase in the frequency and intensity of both of these stressors. Future research should focus on developing mungbean varieties that are more tolerant to UV radiation and drought stress.

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