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# Differential trade-offs between morpho-physiological, antioxidant profile and nutritional qualities of proso millet seedlings under elevated UV-B radiation

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**Amid numerous climate-crisis, elevated UV-B (eUV-B) influences the productivity and quality of common crops; however, its impact is unexplored in nutritionally enriched millet. The present study demonstrates the impact of eUV-B radiation on seedlings of two Proso millet cultivars (TNAU 145 and TNAU 202). We investigated detailed morpho-physiological characteristics, nutritional qualities and biochemical parameters in these cultivars under eUV-B. Our result indicated eUV-B induced germination and biomass accumulation with substantial decrease in chlorophyll and carotenoid and photosynthetic efficiency ($F_v/F_m$) in both the cultivars. TNAU 145 showed significantly higher membrane lipid peroxidation and solute leakage than TNAU 202, indicating greater UV-B susceptibility. Total phenolic content was increased by 81.5% and 73.2% in TNAU 202 and TNAU 145 respectively, although flavonoid content was exclusively increased in TNAU 145 by 31.5%. Significant increase for ROS was recorded in TNAU 202 ($H_2O_2$ by 23% and, $.O_2^-$ by 25.3%) contrary to TNAU 145 ($H_2O_2$ by 29.7% and $.O_2^-$ by 29.5%) whereas antioxidants were increased by 12.5% (SOD), 21.1% (CAT) and 37.5% (APX) in TNAU 202. Moreover, significant increase was observed for total soluble sugar (34%), reducing sugar (13.7%), starch content (16.7%), total protein (3.4%) content and total free amino acid (11.8%) in TNAU 202 reflecting the germination-induced biochemical changes in combination with UV-B radiation. The present study indicated that enhanced antioxidants and bioactive compounds in TNAU 202 attributed to its better performance under eUV-B leading to improved nutritional qualities and biomass accumulation relative to TNAU 145.**

**Keywords** Proso millet, eUV-B, Antioxidant activity, Bioactive compounds, Nutritional quality

UV-B is widely recognized for its adverse effects on agro-economic crops, causing alterations in morphological traits, physiological and biochemical characteristics as well as genetic makeup of various plants[1–3]. The anticipated stratospheric ozone layer recovery reduces UV-B radiation by virtue of Montreal Protocol; however, Kigali Amendment to the Montreal Protocol (2016) listed out certain compounds which contribute directly or indirectly to ozone depletion. Consequently, this leads to influx of Ultraviolet-B (UV-B) radiation (280–315 nm) on earth surface and alter the growth and development of natural and agricultural ecosystems[4]. Sublethal application of UV-B radiation that induce physical or chemical stress can elicit beneficial effects by triggering the accumulation of stress-related compounds such as flavonoids and phenolics[5]. This process is often accompanied by the production of compatible solute and antioxidants as well as secondary metabolites for stress adaptations which has useful health benefits[6,7]. The variability in climate factors along with extreme events such as high temperatures, drought and radiation poses significant challenges to food security. In the view of this, stress-tolerant and superior nutrient rich crops like millet became need of the hour for future agriculture and sustainability[8]. Considering the scenario, the United Nations (UN) General Assembly proclaimed 2023 as the International Year of Millets (IYM2023) as well as ICRISAT declared millet as "future crops". Researchers should employ various strategies to improve the nutritional properties involving stress priming which is treated as an exceptional and reliable method of quality improvement in crops like millet.

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Millets comprise a diverse group of small-seeded grain from *Poaceae* family. Millet is renowned for its resilience, adaptability to diverse agro-climatic condition and its exceptional nutritional value stand it out as vital means for nutritional food security subject to climate change across the world[9]. Millet outperforms grains like rice and wheat for the fact in thriving under marginal growing conditions while offering superior nutritional benefits. In India, millet is classified based on the grain size, with larger one regarded as major millets and the smaller referred to as minor or small millets[10]. Pearl millet, finger millet, sorghum are regarded as major millets, while proso, little, barnyard, foxtail and kodo millet(s) are classified as minor millets[11]. Major millets are produced in larger quantities than minor millet due to their popularity. Interestingly, small sized minor millets are nutrient-dense, non-acid forming and easily digestible containing high levels of protein, fiber and essential minerals that can fight the hidden hunger issue of developing countries[12]; hence treated as nutri cereals. In the present investigation, we utilized minor millet proso because of its highest protein content (12.5gm/100gm) among all cereals[10], ancient global adaptability, versatile use like food or animal-feed, high drought tolerant model crop due to short life cycle, rich nutritional property and genetic simplicity.

Proso millet (*Panicum miliaceum* L.) is a C4 drought tolerant crop that grows to a height of 45–130 cm and has a shallow, fibrous root system[13]. It is a short season (60–90 days) crop with low water requirement and can grow under extreme soil and climate condition, hence useful for contingency farming[14,15]. Many reports suggested intake of this plant significantly increases the plasma adiponectin and cholesterol levels while markedly retarding the levels of insulin compared to type 2 diabetic mice having casein diet[16,17], protective effects against D-galactosamine-induced mice liver injury[16]. Proso millet-based food has observed to downregulate STAT3 signalling pathway, a family of transcription factors crucial in promoting uncontrolled cell proliferation, angiogenesis and resistance to apoptosis in cancer cells therefore preventing cancer[18,19]. Proso millets exhibit high total antioxidant capacity, attributed to their elevated levels of carotenoids and tocopherols, respectively[20]. Additionally, Proso millet contains 9.5–17% protein comparable to or higher than the protein content of wheat and other cereals. Protein content is rich due to essential amino acids like methionine, isoleucine and leucine[21]. "Shiloh Farms" and "To Your Health" are two famous US based retail brand started the buzz of commercial sprouted millet grain or seedling products as a new addition to food industry with enhanced nutritional value and improved nutritional absorption on the account of the fact that germination improve the availability of reducing sugar, free amino acid, phenolic compound and antioxidant capability[22].

Plants have developed physiological and biochemical mechanisms to cope with stress by the increased production of protective metabolites that can mitigate stress. These adaptive responses offer opportunities to enhance health-promoting compounds in stressed plants. Consequently, when germination under standard conditions fails to achieve optimal nutrient levels, applying environmental stress treatments as an elicitor can improve the functional biochemical profile and antioxidant potential of sprouted grains[22]. Physical treatments as elicitors are explored for their impact on the nutritional composition of seedlings at the early stage of development. These include UV light, high hydrostatic pressure, cold shock and cold plasma respectively[23–25]. Henceforth, while UV-B radiation can harm plant tissues, it has also been shown to enhance the accumulation of antioxidants and UV-protective compounds in certain plants[26]. Proso millet being climate resilient, nutrient rich crop with significant potential to contribute to food and nutritional security, presents a promising crop for such studies. Therefore, the present study was undertaken to address this knowledge gap by evaluating the morpho-physiological changes as well as nutritional and antioxidative properties of two high yielding proso millet varieties TNAU 145 and TNAU 202, that will be beneficial for breeding programs aimed at developing UV-B-resilient, nutritionally superior millet varieties.

## Results

### Germination and morphological results

GP of TNAU 145 was significantly increased to 12.19% ($p \leq 0.05$) under eUV-B; however, in TNAU 202 the increment in nonsignificant by 9.3% relative to control (Fig. 1A). GV increased upon eUV-B exposure for both the cultivar and the speed of germination was faster in TNAU 145 (10.3%) than TNAU 202 (7.2%) (Fig. 1B). Results of the two-way ANOVA depicted that Cv and T was significantly affected the GP whereas T only was influential for GV (Table 1). The SL significantly increased to 23.2% ($p \leq 0.01$) and 33.3% ($p \leq 0.05$) for TNAU 145 and TNAU 202 respectively (Fig. 1C) where as RL increased significantly by 28.8% ($p \leq 0.01$) in TNAU 202 (Fig. 1D). Maximum increase in biomass was observed in eUV-B exposed plants and increase in biomass was more pronounced in TNAU 202 by 32.4% ($p \leq 0.01$) than TNAU 145 (31.8%, $p \leq 0.01$) (Fig. 1E). SL, RL and TB was significantly influenced by Cv, T and Cv × T (Table 1).

### Photosynthetic pigments

Photosynthetic pigment chlorophyll reduced significantly by 35% ($p \leq 0.001$) and 19% ($p \leq 0.001$) respectively in TNAU 145 and TNAU 202 under eUV-B (Fig. 2A). Similar trend was observed for carotenoid (Fig. 2B). Reduction of these pigment content was found higher in TNAU 145 than TNAU 202 under eUV-B at seedling stage. It was observed that eUV-B induced the biosynthesis of anthocyanin in TNAU 202 reflected the significant increase by 19.6% ($p \leq 0.001$) relative to control; however, the decrease in anthocyanin content was observed in TNAU 145 by 18.4% due to eUV-B exposure (Fig. 2C). Result of two-way ANOVA showed that under eUV-B photosyntheticpigments of both the cultivar significantly affected by Cv, T and Cv × T. However, anthocyanin pigment varied significantly due to Cv and Cv × T (Table 1).

### Bioactive compound

Significant increase of phenol content by 81.5% ($p \leq 0.001$) was observed in TNAU 202 compared to TNAU 145 (73.2%, $p \leq 0.01$) (Fig. 2D). Significant increase in total flavonoid content by 31.5% ($p \leq 0.001$) was found in TNAU 145; however, reduction in flavonoid by 13.8% ($p \leq 0.05$) was observed in TNAU 202 under eUV-B

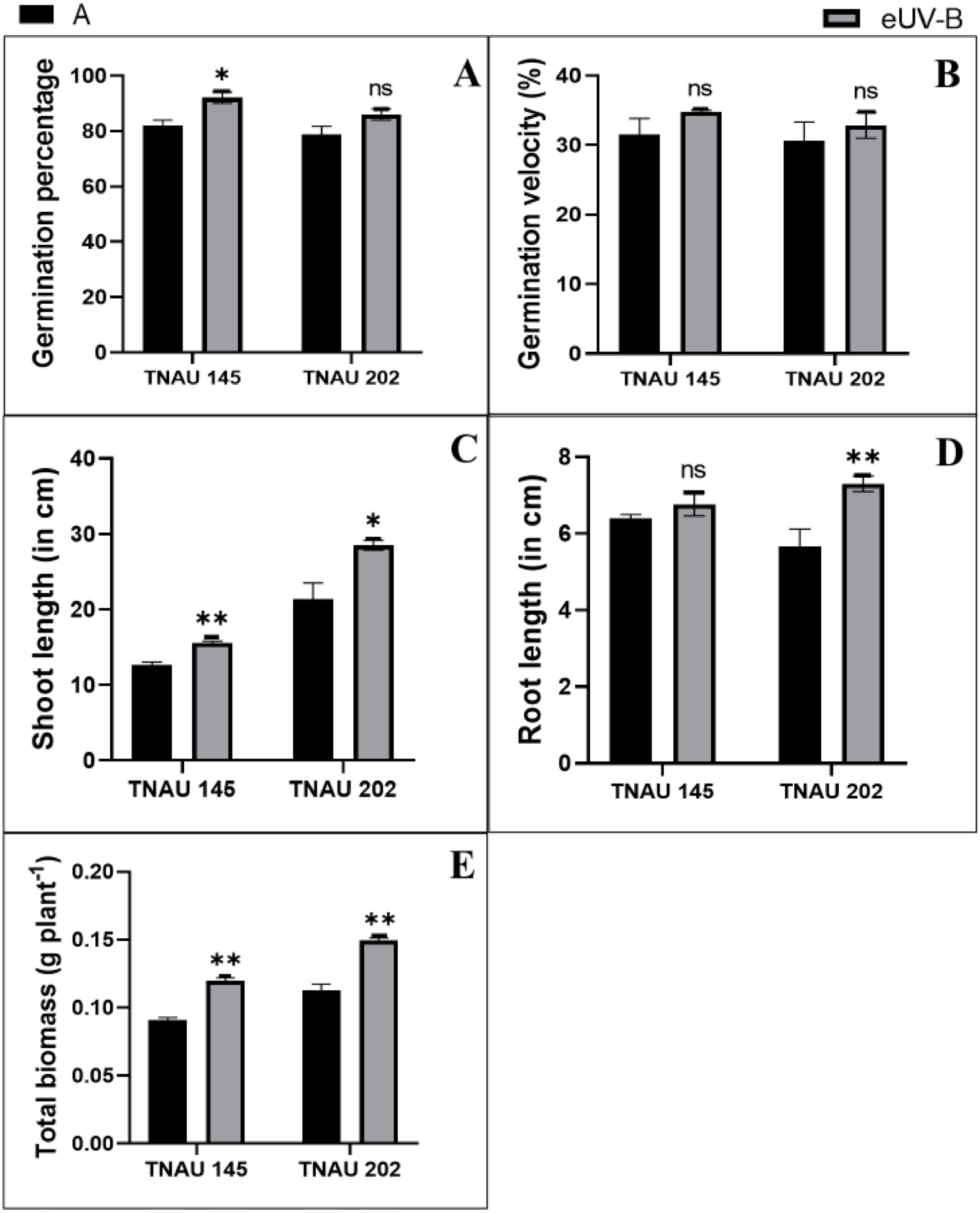

**Fig. 1**. Effects of eUV-B radiation on germination values and morphological parameters in Proso millet cultivar TNAU 145 and TNAU 202. (**A**) Germination percentage (GP); (**B**) Germination velocity (%) (GV); (**C**) Shoot length (SL); (**D**) Root length (RL) and (**E**) Total biomass (TB). Values shown as bars are mean ± SD. Level of significance: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ns: Not significant, A: ambient and eUV-B: elevated UV-B.

| Parameters | $C_v$ | T | $C_v \times T$ |
|---|---|---|---|
| GP | ** | *** | ns |
| GV | ns | * | ns |
| SL | *** | * | * |
| RL | ns | *** | ** |
| TB | *** | *** | * |
| Total chlorophyll | *** | *** | *** |
| Carotenoid | *** | *** | *** |
| Anthocyanin | *** | ns | * |
| Phenol | *** | *** | ** |
| Flavonoid | *** | ns | *** |
| $H_2O_2$production | *** | *** | ns |
| $^{\bullet}O_2^-$ production | *** | *** | ns |
| LPO | ** | ** | ns |
| Solute leakage | *** | ns | ns |
| SOD | *** | ns | ns |
| CAT | *** | *** | ** |
| APX | *** | ** | ns |
| Protein | *** | *** | *** |
| $F_0$ | ns | *** | ns |
| $F_m$ | ns | *** | ns |
| $F_v$ | *** | *** | ns |
| $F_v/F_m$ | *** | ** | ns |
| TFAA | *** | *** | ns |
| TSS | *** | *** | ** |
| RS | ns | * | ns |
| SC | ns | * | ns |

**Table 1**. Statistical analysis of Two-way ANOVA showing level of significance for selected parameters of proso millet cultivars (year: 2024) grown in ambient and elevated UV-B. Note: T, treatment; Cv, cultivar. $^*p \le 0.05$; $^{**}p \le 0.01$, $^{***}p \le 0.00$; ns, not significant.

(Fig. 2E). Result of Two-way ANOVA depicted that phenol and flavonoid content indicated significant variation due to Cv, T and Cv × T except flavonoid content under different treatment (Table 1).

## Physiological parameters

eUV-B resulted in significant increase in $F_0$ to 17.7% ($p \le 0.05$) in TNAU 145 than TNAU 202 (12.8%) (Fig. 3A). Result showed significant decrease in $F_m$, $F_v$ and $F_v/F_m$ to 6% ($p \le 0.01$), 16% ($p \le 0.01$) and 10.6% ($p \le 0.01$) in TNAU 145 while $F_m$ value significantly decreased in TNAU 202 so as $F_v$ and $F_v/F_m$ (Fig. 3B, C and D). The major impact of eUV-B for TNAU 145 occurred in the J-I phase which is a component of OJIP transient, however J-I phase was least affected in TNAU 202 indicating less damage to PSII reaction centers (Fig. 3E and F). $F_0$, $F_m$, $F_v$ and $F_v/F_m$ were significantly affected by treatment only whereas $F_v$ and $F_v/F_m$ showed significant variation in Cv under eUV-B exposure (Table 1).

## ROS formation, solute leakage and lipid peroxidation

eUV-B trigger ROS production in term of $H_2O_2$ and $^{\bullet}O_2^-$ in the foliar tissue of the test cultivar. Result showed the significant production of $H_2O_2$ and $^{\bullet}O_2^-$ by 23% ($p \le 0.05$) and 25.3% ($p \le 0.05$) respectively in TNAU 202. $H_2O_2$ and $^{\bullet}O_2^-$ generated in TNAU 145 by 29.7% ($p \le 0.05$) and 29.5% ($p \le 0.01$) respectively (Fig. 4A and B). Moreover, the significant increase in $H_2O_2$ and $^{\bullet}O_2^-$ in both the test cultivar was accompanied with increase in MDA content and solute leakage. MDA content was recorded 61.5% ($p \le 0.05$) in TNAU 145 and 52.4% in TNAU 202 under eUV-B. The solute leakage represented by electrolyte leakage index (ELI) was measured 25.2% for TNAU 145 and 21% ($p \le 0.05$) for TNAU 202 relative to control (Fig. 4C and D). The result of two-way ANOVA showed significant effect under eUV-B Treatment (T), Cv on the production of $H_2O_2$ and $^{\bullet}O_2^-$ and lipid peroxidation. However, solute leakage showed significant variation due to Cv only (Table 1).

## Antioxidant defense system

SOD, APX and CAT activities were significantly increased in TNAU 202 under eUV-B treatment. SOD activity increased by 12.5% ($p \le 0.05$) in TNAU 202 and 4.7% in TNAU 145 (Fig. 5A). CAT and APX activities increased by 21.12% ($p \le 0.01$), 37.5% ($p \le 0.05$) for TNAU 202 and 18% and 10.5% for TNAU 145 respectively (Fig. 5B and C). Two-way ANOVA significantly varied due to T, Cv and their interaction on CAT activity and protein content. Additionally, SOD showed significant variation in cultivar only and Cv and T indicated significant effect on APX activity (Table 1).

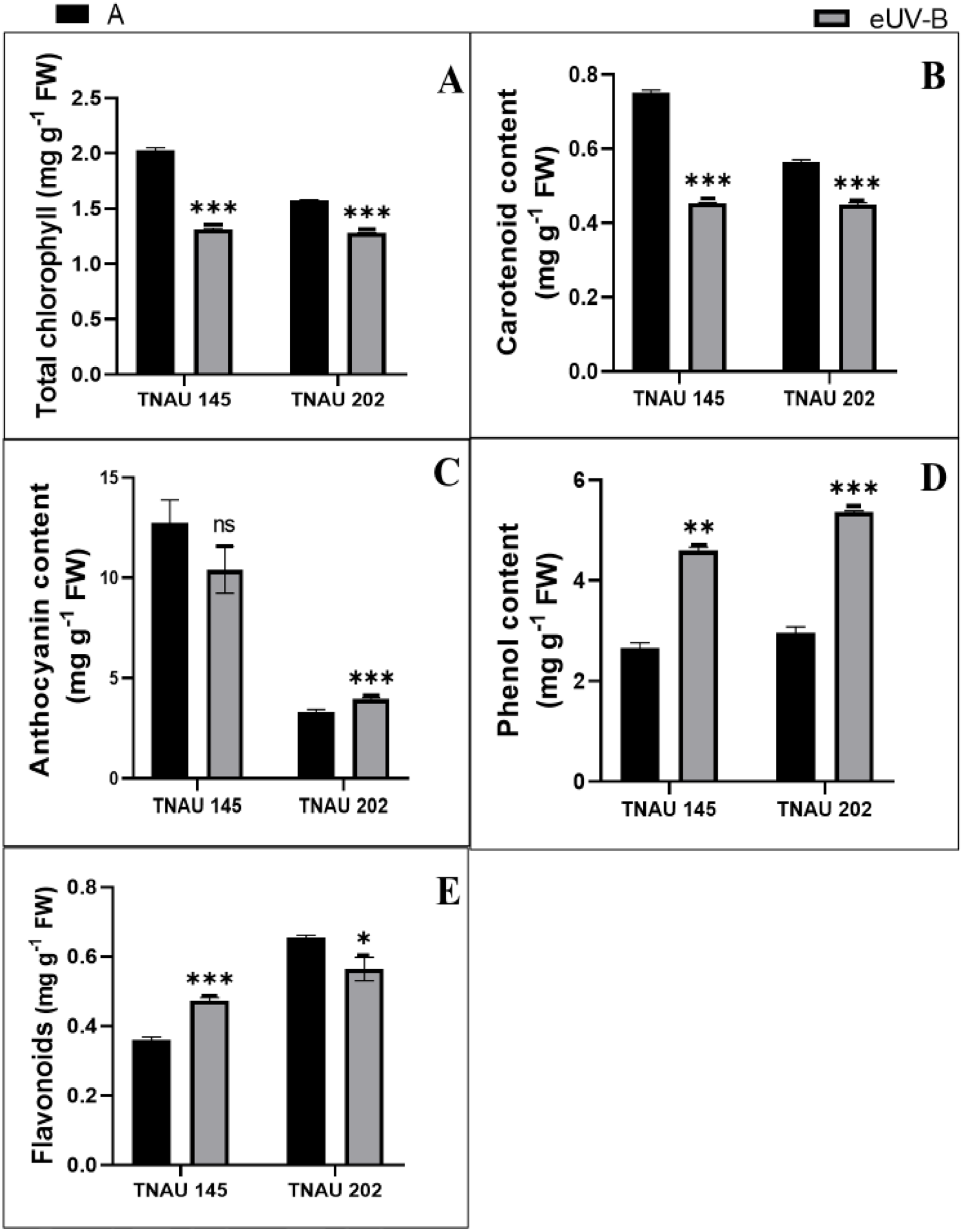

**Fig. 2**. Effects of eUV-B radiation on pigments and bioactive compounds in Proso millet cultivar TNAU 145 and TNAU 202. (**A**) Total chlorophyll; (**B**) Carotenoids content; (**C**) Anthocyanin content; (**D**) Total phenol content and (**E**) Flavonoids. Values shown as bars are mean ± SD. Level of significance: $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, ns: Not significant, A: ambient and eUV-B: elevated UV-B.

## Nutritional quality

Significant increase in TFAA content of both the test cultivar TNAU 145 (10.13%, $p \leq 0.05$) and TNAU 202 (11.8%, $p \leq 0.01$). Similar trend in the increment of TSS, RS and SC was evident in both the test cultivar and the biosynthesis of sugar and amino acid was more pronounced in TNAU 202. Significant decline in protein content by 13.6% ($p \leq 0.001$) was observed in TNAU 145 under eUV-B in contrast to significant increment of protein

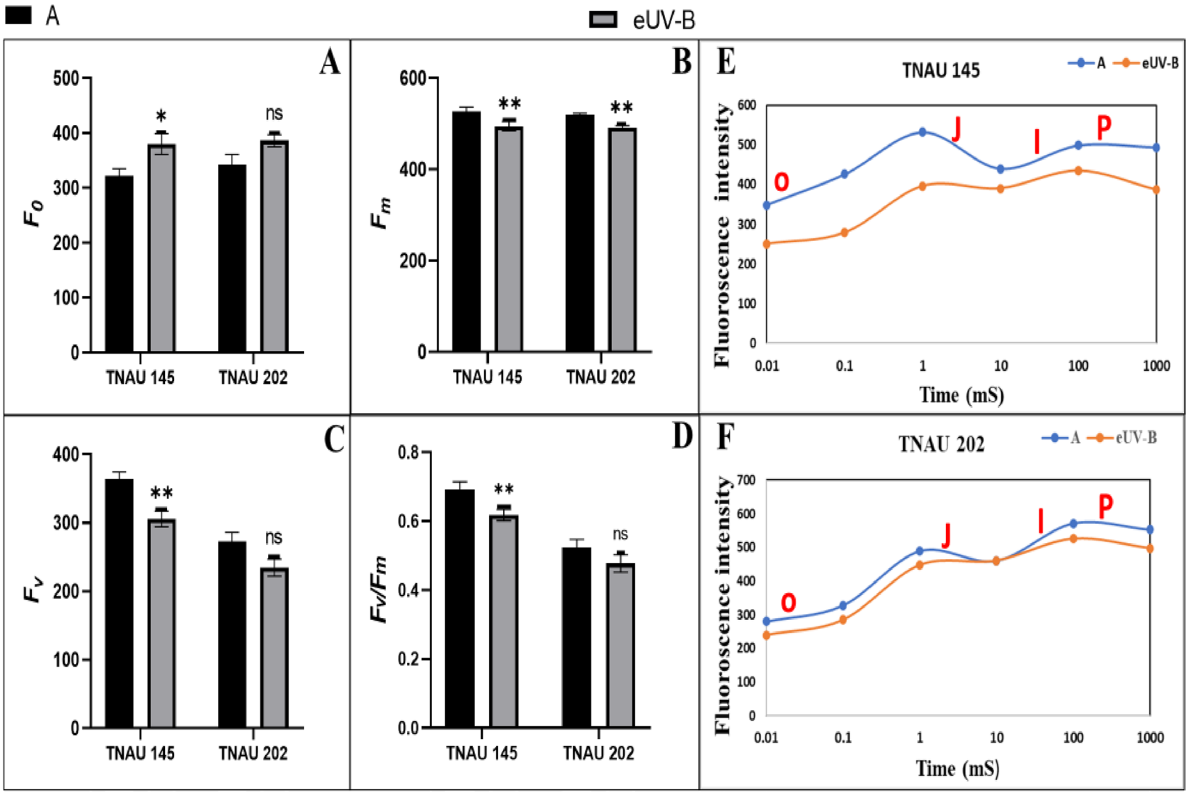

**Fig. 3**. Effects of eUV-B radiation on physiological parameters in Proso millet cultivar TNAU 145 and TNAU 202. (**A**) $F_0$; (**B**) $F_m$; (C) Fv; (**D**) $F_v/F_m$ values; (**E**) Chlorophyll fluorescence OJIP curve of TNAU 145 and (**F**) Chlorophyll fluorescence OJIP curve of TNAU 202. Values shown as bars are mean ± SD. Level of significance: *$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$, ns: Not significant, A: ambient and eUV-B: elevated UV-B.

content by 3.4% ($p \le 0.05$) in TNAU 202 (Fig. 6B). Result of two-way ANOVA demonstrated significant variation due to the effect of T and Cv on TFAA whereas TSS significantly varied due to Cv, T and Cv × T. In case of RS and SC significant variation is only evident in T (Table 1).

## Discussion

In the present study, proso millet seed germination values (GP and GV) were positively influenced by eUV-B (Fig. 1A and B). Our result aligns with the finding of[27]in mung bean. Additionally[28], depicted better germination rate of several seeds under the influence of eUV radiation. eUV-B stimulated seed germination due to high energy photon with a range of 280–320 nm than visible light (400 nm) as UV-B radiation has stronger effect on surface or near-to surface level of plants. The high energy photon may increase permeability of seed to water and gases by breaking the seed coat which leads to faster germination. Application time and elevated dose of UV-B exposure may lead to nonsignificant germination values of both of our test cultivars as supported by[29]. Other scientific reports suggested the critical evidences of facilitated germination by the incidence of high energy radiation is probably due to activation of RNA or protein synthesis as well as modulation of plant hormonal pathways in the early stage of germination after irradiation prime[30,31]. An alternate prospect of enhanced germination is eUV-B induced biosynthesis of ethylene which is coupled with generation of signalling molecule e.g. ROS that is otherwise involved in ABA/GA balancing to lead many developmental processes of plant life cycle seed dormancy alleviation and germination[32,33]. Ozone, another contributor of ROS has been demonstrated to promote seed germination and stimulate the expression of seed proteins[34,35].

Significant increase in plant growth parameters like SL, RL and TB under eUV-B were observed in our study (Fig. 1A, B and C) which is similar to the finding of[36] in groundnut and mung bean[37]. suggested the similar trend of growth pattern in Bengal gram under UV radiation at 253 nm. UV-B induced germinated seedling showed increase in length and biomass because of facilitated mitotic division under supplemental UV light[37,38]. The rise in SL may be linked with higher IAA accumulation which absorbs the UV radiation and supported the modified growth pattern. Higher incidence of UV radiation on plants showed reduced growth parameters and it depicted the harmful impact from 8 kJ/m$^2$/d of illumination[39]. In our study, this eUV-B dose may be treated as optimum level that promote seedling growth and biomass accumulation. A study of[40] found a gene *GmILPA1* in soybean which upregulates plant height and UV-B light incidence induces the accumulation of *GmILPA1*. Translocation and utilization of photosynthates towards the shoot tip region of eUV-B treated plants resulted in enhanced plant height[41]. Elevated UV-B under PAR (low and high) may influence increased biomass production. UV-B

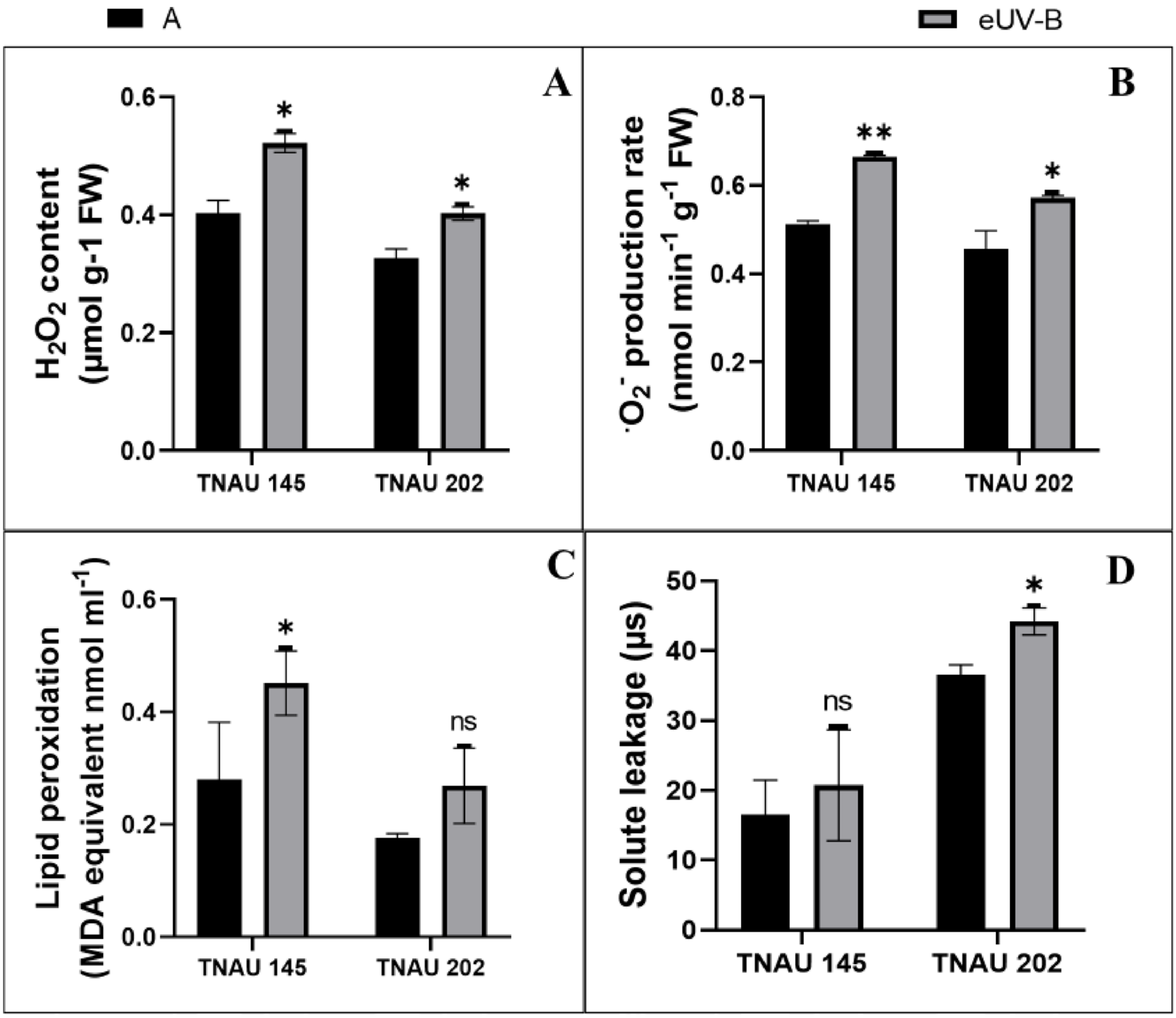

**Fig. 4**. Effects of eUV-B radiation on ROS production and membrane damage in Proso millet cultivar TNAU 145 and TNAU 202. (**A**) $H_2O_2$ content; (**B**)$^{\cdot}O_2^-$ production rate; (**C**) Lipid peroxidation (MDA content) and (**D**) Solute leakage. Values shown as bars are mean ± SD. Level of significance: $*p \le 0.05$, $**p \le 0.01$, $***p \le 0.001$, ns: Not significant, A: ambient and eUV-B: elevated UV-B.

radiation influences auxiliary branch development and therefore promote branching as evident in *Mentha spicata* so as to facilitate leaf development that may acquire stress acclimatization[42] as well as biomass accumulation.

The degradation or reduction of photosynthetic pigments occurred in the present experiment under eUV-B (Fig. 2A and B) exposure has a clear indication of detrimental effect of UV-B radiation on photosynthetic machinery. eUV-B primarily affect the photosynthetic apparatus and alternate the $CO_2$ diffusion which ultimately hampers the photosynthetic rate[43]. In the early stage of development, supplemental UV radiation may inhibit the biosynthesis of precursors like protochlorophyllide and protochlorophyll[44]. Cultivar TNAU 145 showed relatively more degradation of chlorophyll and carotenoid (Fig. 2A and B) due to generation of more ROS (Fig. 4A and B) that is the main reason of impaired pigment architecture under eUV-B radiation[45]. UV-B radiation reduces the leaf area as well as enable the development of thicker palisade containing higher amount of chlorophyll per unit area in cucumber cotyledon[46]. The reduced leaf area is more pronounced than thicker palisade containing chlorophyll suggested noticeable decrease in photosynthetic pigments under UV-B radiation[4748] reported initial increase in Chlorophyll content under eUV-B treatment and subsequent decrease due to the degradation of carotenoid, which acts as an efficient quencher against UV-B radiation. TNAU 145 showed decrease in anthocyanin where as TNAU 202 showed opposite trend (Fig. 4C). This result indicated that anthocyanin absorbed the harmful radiation of UV rays so as to protect the photosynthetic apparatus and prevent more degradation of photosynthetic pigments in TNAU 202 compared to TNAU 145. Increase in anthocyanin pigment of TNAU 202 under eUV-B is in agreement with the findings of[49,50]. UV-B induced *COP1/HYH/HY5* signalling pathway with increased $H_2O_2$ which is involved in biosynthesis and accumulation of anthocyanin pigment as a part of defense mechanism coupled with *UVR8* expression in radish sprout[51].

Total phenol content was significantly enhanced under eUV-B radiation in both the cultivars; however total flavonoid content was found high only in TNAU 145 (Fig. 4D and E). This finding is supported by the result of[5,52]. UV-B radiation activates a key enzyme phenylammonia lyase (PAL) which facilitate the accumulation

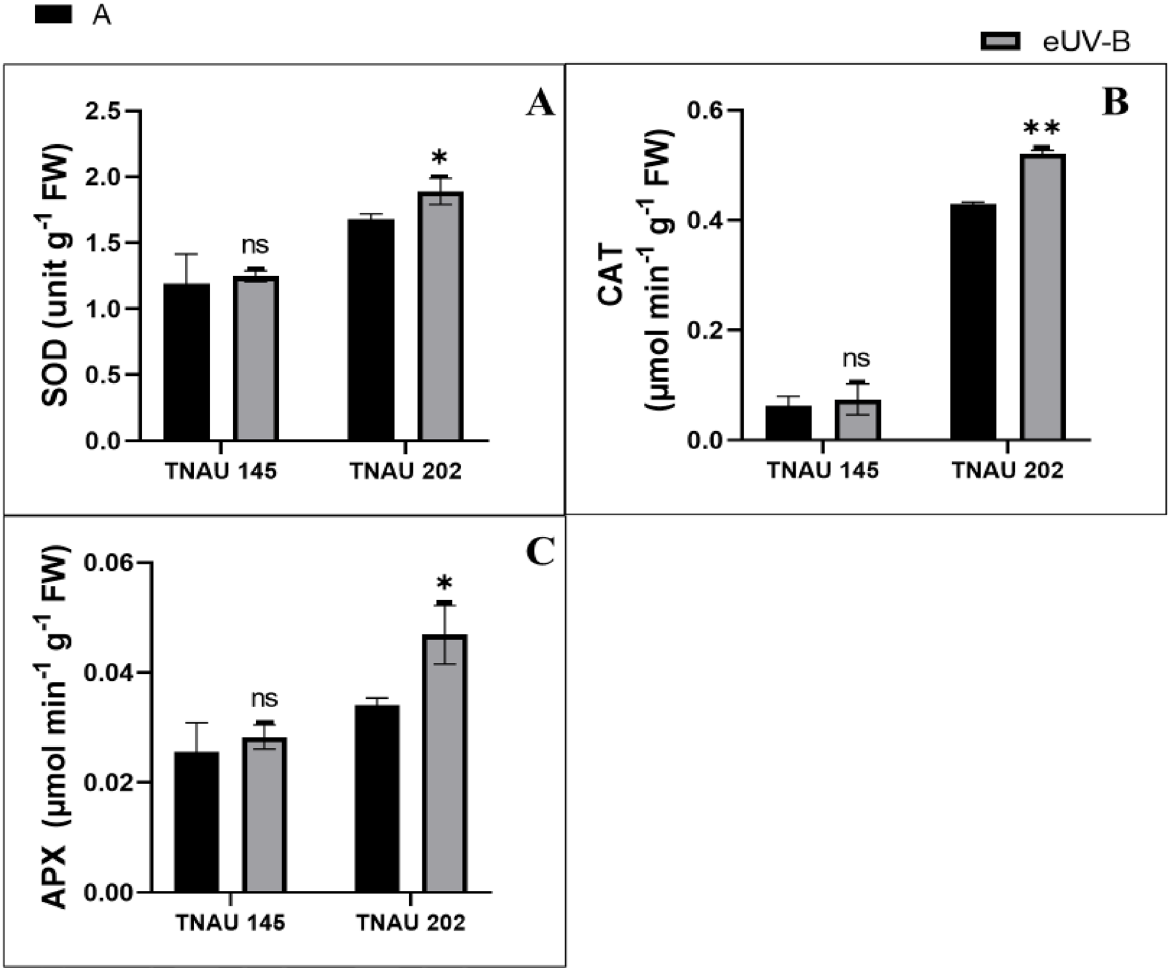

**Fig. 5**. Effects of eUV-B radiation on antioxidative defense activities in Proso millet cultivars TNAU 145 and TNAU 202. (**A**) SOD activity; (**B**) CAT activity and (**C**) APX activity. Values shown as bars are mean ± SD. Level of significance: $*p \le 0.05$, $**p \le 0.01$, $***p \le 0.001$, ns: Not significant, A: ambient and eUV-B: elevated UV-B.

of phenolic compound promoting antioxidant capacity in barley and wheat[53]. Upon eUV-B treatment, total flavonoid content significantly increased in TNAU 145; however, in TNAU 202 decrease in flavonoid content occur along with the increase in anthocyanin pigment. High flavonoid content protects the mesophyll tissue from photo-oxidative damage due to eUV-B in *Indigofera tinctoria* seedling[48]. An interesting observation was reported in brown rice where anthocyanin and flavonoid content was high in the initial stage of growth and development however 8 to 16 days after germination the flavonoid content gradually decreased and anthocyanin content was stable[54]. The possible reason behind this process is redirection of metabolic flux and optimal resource allocation towards anthocyanin biosynthesis to prioritise photoprotection under eUV-B condition as anthocyanin and flavonoid share same phenylpropanoid pathway.

Chlorophyll fluorescence is commonly utilized to assess stress-induced modifications in the photosynthetic machinery. In the present investigation, both the test cultivar displayed an $F_0$ increase (Fig. 3A) and decrease in $F_m$ and $F_v/F_m$ (Fig. 3B, C and D) upon eUV-B treatment. This increase in $F_0$ indicates an irreversible damage to PSII reaction center due to separation of LHCII and hindrance in the ETC, which consequently leads to reduction in $F_v/F_m$ ratio[55]. OJIP analysis also reflected that damage to PSII reactions centers were less pronounced in TNAU 202 as compared to TNAU 145. Reduction of $F_v/F_m$ is more evident in TNAU 145 which might be due to damage to PSII reaction centres by slow quenching, thus reducing the quantum efficiency of PSII as supported by[56]. Additionally, disruption of linear flow of electrons ($e^-$) beyond PSII is also impacted by the enhanced UV-B radiation, which leads to decrease in RuBP regeneration, which in turn lowers the rate of Rubisco carboxylation and finally the photosynthetic rate[57]. These results were also supported by the findings of[58] due to light-dependent inactivation of PSII reaction centres in spinach and amaranthus. Even minimal doses of UV radiation led to shift in the ontogenetic sequence for the developmental progression of photosynthetic capacity[59].

Similar to our study, ROS generated by eUV-B inflicts PSII damage including D1/D2 proteins, oxygen evolving complex, and other components associated with the photosystem II and simultaneously inactivate LHC II[60]. In the present investigation, eUV-B induced ROS resulted in lipid peroxidation, membrane damage due

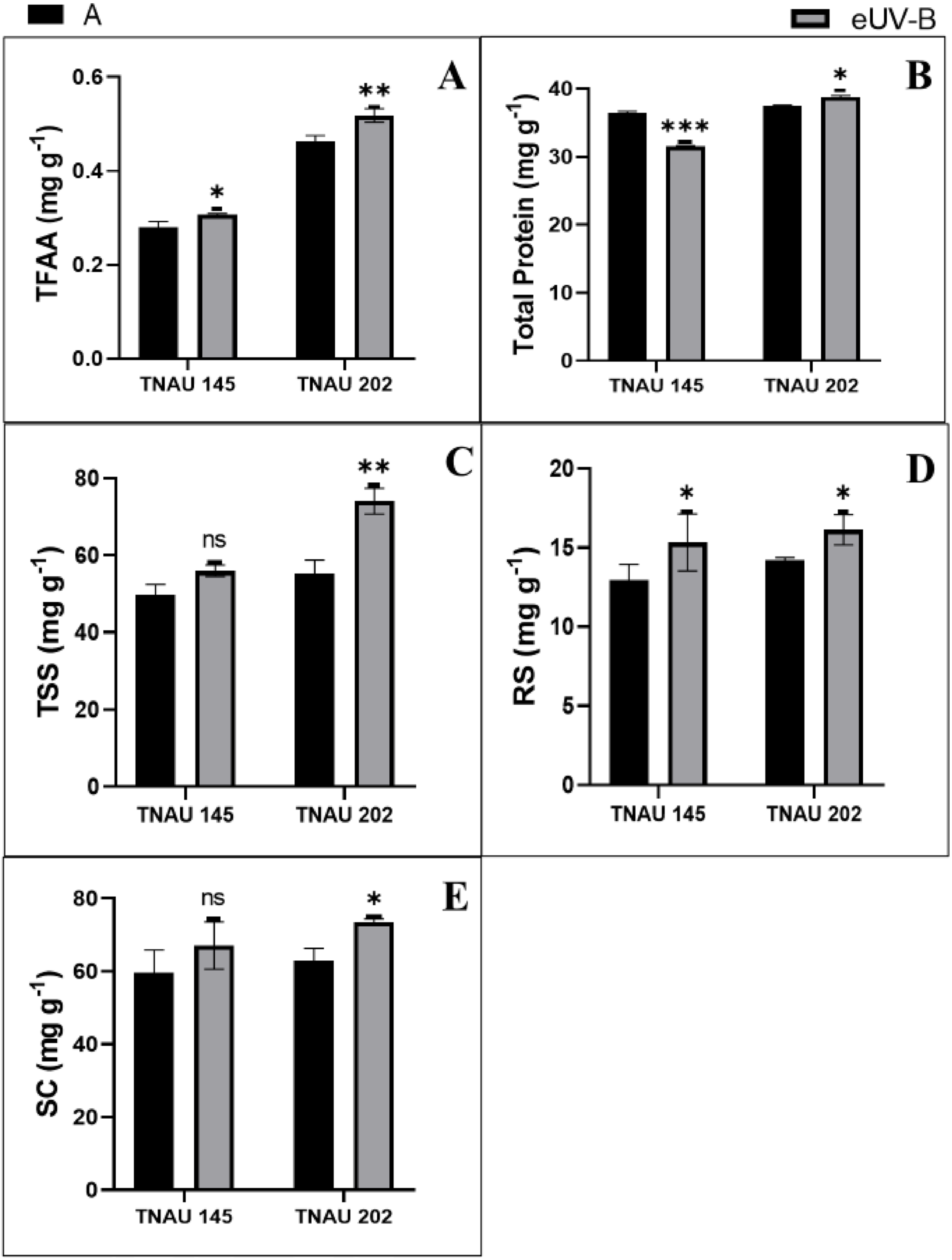

**Fig. 6**. Effects of eUV-B radiation on nutritional qualities in Proso millet cultivars TNAU 145 and TNAU 202. (**A**) Total free amino acid (TFAA); (**B**) Total protein; (**C**) Total soluble sugar (TSS); (**D**) Reducing sugar (RS) and (**E**) Starch content (SC). Values shown as bars are mean ± SD. Level of significance: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ns: Not significant, A: ambient and eUV-B: elevated UV-B.

to MDA production and subsequent solute leakage in both the cultivar. Similar results have been observed in tropical mung bean as supported by the reports of[61] as well as[62] described parallel result in wheat. Furthermore, higher production of ROS in TNAU 145 is responsible for more lipid peroxidation and electrolyte leakage as compared to TNAU 202 (Fig. 4C and D). ROS-induced loss of member integrity triggers a cascade irreversible damage including osmotic imbalance, destroyed membrane permeability and stability[63]. $^{\bullet}O_2^-$ causes immediate and localized damage in plants under UV-B stress due to its high reactivity, hence concurrently converted to $H_2O_2$ by an enzymatic antioxidant, SOD to abolish the harmful effect of UV-B stress; hence SOD is regarded as the first defense barrier against the oxidative stress[64]. SOD plays crucial role in converting the $^{\bullet}O_2^-$ into the less toxic $H_2O_2$, which is subsequently broken down into water ($H_2O$) by APX, catalase and total peroxidase[65]. $H_2O_2$ is comparatively less reactive but more damaging because of its stability and accumulation in the subcellular spaces causing oxidative stress. In our study, $^{\bullet}O_2^-$ production rate is slightly higher than $H_2O_2$ in both the test cultivar (Fig. 4C and D). Henceforth, the availability or activity of SOD found relatively less than CAT and APX (Fig. 5A, B and C) as SOD is involved in neutralizing the $^{\bullet}O_2^-$ and gradually became saturated, which is confirmed by the finding of[66].

A significant increase in enzymatic antioxidative defense activities (SOD, CAT and APX) was observed in TNAU 202 under eUV-B treatment which leads to lower accumulation of ROS (Fig. 5A, B and C). Antioxidants like SOD, CAT and APX enhanced during the initial stage of plant development under eUV-B exposure to protect the plants by scavenging the ROS and maintain the redox homeostasis of the cell[67,68]. SOD in TNAU 202 is the main scavenger of superoxide radicals[61]. Changes in the activities of enzymes possibly resulted from the coordinated interactions and cross-talk among enzyme and their end products, which collectively regulate the overall defense mechanism[69]. Numerous reports suggested increase in APX and CAT activity rapidly under low and high UV-B radiation subsequent to SOD under eUV-B[70,71]. Studies have shown that chloroplast APXs are inhibited in the presence of $H_2O_2$ when ascorbic acid levels are depleted, indicating that UV-B radiation may induce ROS-mediated disruptions in the antioxidative defense system[72]. In the present investigation, TNAU 202 exhibit higher APX activity compared to CAT (Fig. 5B and C) which is validated by[73]. Additionally, he explained that APX shows considerably stronger affinity for $H_2O_2$ than CAT, utilizing ascorbate as a specific electron donor to convert $H_2O_2$ into $H_2O$. Enhanced expression of cytosolic, chloroplastic and peroxisomal APX boosts plant's resilience to abiotic stresses such as chilling, drought, salinity, heat and UV-B radiation[74].

In the present study, TNAU 202 seedlings displayed higher of protein content under eUV-B; however, protein degradation was evident in TNAU 145 (Fig. 6B). Phenol content was explicitly high in TNAU 202 (Fig. 2D) which is an important response of plants against abiotic stress leads to biosynthesis of protein as evident by the finding of[75]. The possible reason of increased protein content in TNAU 202 under UV-B radiation suggests repair of plasma membrane, allocation of proteins towards defense-related functions, enhanced synthesis of heat shock proteins and the production of regulatory proteins such as transcription factor[76]. The nonsignificant increase of MDA content in TNAU 202 (Fig. 4C) along with higher protein content was obvious which may be engaged in repair of damaged membrane due to eUV-B radiation[77]. In TNAU 145 resulted in degradation of protein (Fig. 6B) and possible factor contributing to protein degradation is UV radiation induced ROS production, which can modify and damage amino acids, while disulphide bonds strongly absorb UV light leading to their breakdown as reported by[78].

Seedling of proso millet tested cultivars showed positive result for the nutritional parameters in terms of TFAA, TSS, RC and SC under eUV-B (Fig. 6A, C, D and E). During initial stages of development plants exhibit higher amino acid content could be attributed to the activation of various enzymes in relation to germination[79]. TNAU 202 showed significantly higher TFAA over TNAU 145 which directly employ that the quality, content, solubility and digestibility of the protein in the seedling of TNAU 202 is better than TNAU 145 (Fig. 6A). Furthermore, the outcomes of the present investigation revealed the increase of TSS, RS and SC in seedlings of both the test cultivar which is supported by the findings of[80] which described the accumulation of carbohydrate in plant tissues is regarded as mechanism for osmotic adjustment during stress conditions and may serve as respiratory substrates or osmoticum. An additional investigation by[81] reported the increment in TSS under UV-B seed treated maize hybrids which is similar to the present study. Despite the adverse impact of UV-B on photosynthetic activity, the soluble carbohydrate content remained unaffected in cucumber suggesting stable primary metabolite (glucose, fructose and sucrose) content which can deliver respiratory energy for protection, signalling processes, structural maintenance and repair mechanisms[82]. Studies on white clover suggested increase in sugar content in both leaves and root, including TSS, RS and starch which is corroborated with our result[83]. Lowering of RS content of TNAU 202 than TNAU 145 (Fig. 6D) during germination or at the early stages of plant development might be due to diversion of RS into metabolic functions rather than expression of genes involved in development during germination. Meanwhile cellular oxidation increased under eUV-B leads to generation of more ROS that might contribute to oxidation of RS and its gradual decrease[84]. Significant increase in SC of TNAU 202 (Fig. 6E) observed in the present study may be due to retardation in starch breakdown to sugar due to degradation of α-amylase, β-amylase, starch phosphorylase and amylolytic enzymes as a result of UV radiation exposure[85,86,87]. observed an increase in protein, starch and soluble sugar content in purple potato subjected to low UV-B radiation. Several evidence have implicated that increased sugar content was likely attributed to the sufficient nutrient availability in the soil provided during this study[88]. Our result showed an overall increase in free amino acid and sugar which is corroborated with the finding of[89] in *R. chrysanthum* which described that germination in proso millet may enhance free amino acid, total sugar, lysine and tryptophan by simultaneously declining the starch content.

## Conclusion

eUV-B radiation enhanced the seed germination values in TNAU 145. However, total biomass accumulation was positively influenced by eUV-B in TNAU 202 as compared to TNAU 145. Total chlorophyll and carotenoid content

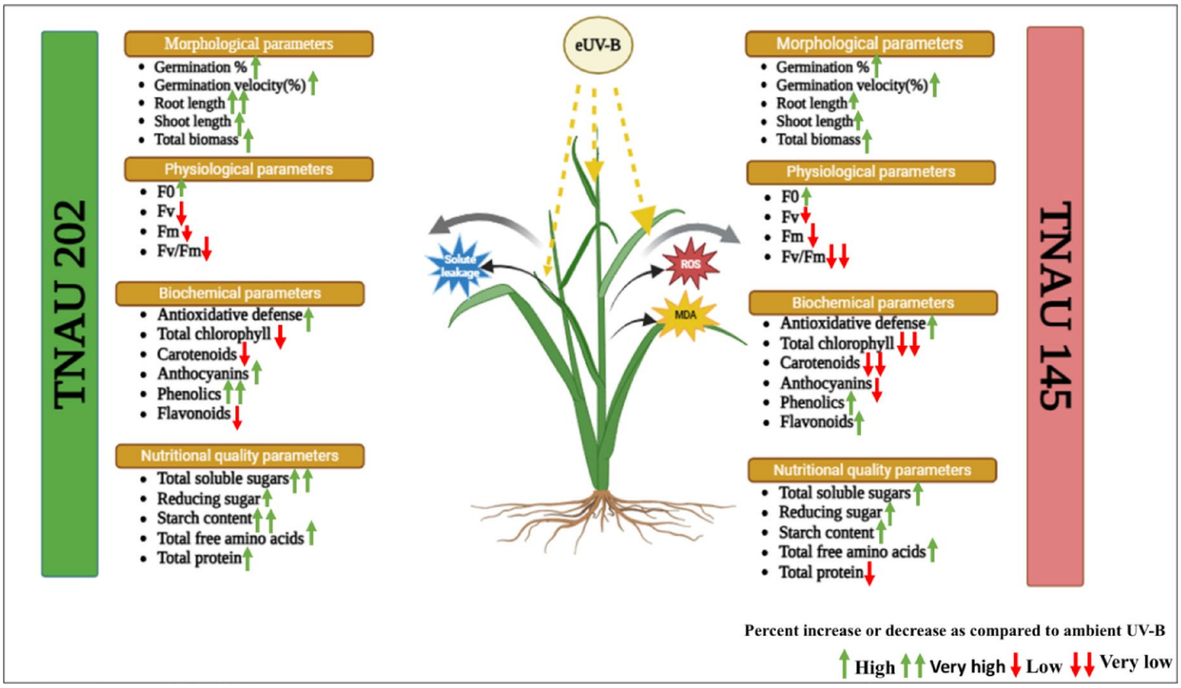

**Fig. 7**. Morphological, Physiological, and Biochemical responses of proso millet cultivars under elevated UV-B (eUV-B) radiation.

| Month/year | | September, 2024 |
|---|---|---|
| Rainfall (mm) | | 567.9 |
| Mean temperature (8 °C) | Max. | 35.4 |
| | Min. | 34.2 |
| Relative humidity (%) | Max. | 100 |
| | Min. | 73 |
| Sunshine (h) | | 5.4 |

**Table 2**. Meteorological variables at the experimental site during the study period.

significantly decreased which led to reduced photosynthetic rate as eUV-B seriously affected the photosystems and chlorophyll architecture. It is clearly evident from the result that eUV-B induced generation of ROS in TNAU 145 and TNAU 202 which led to membrane damage and also solute leakage were more pronounced in former than later. This incidence triggers the antioxidant defense system of plants to mitigate the ROS by enhancing SOD, CAT and APX activities and noticeable increase in antioxidants were more in TNAU 202. Germination induced seedling growth under eUV-B resulted in the elevation of nutritional qualities in terms of protein, total free amino acid, total soluble sugar, reducing sugar and starch content. The overall differential responses of proso millet cultivars under eUV-B has been highlighted in Fig. 7. Therefore, TNAU 202 can be utilized for areas experiencing high influx of UV-B radiation by maintaining its growth as well as nutritional aspects. Therefore, eUV-B radiation to some extent can be used as elicitor of plant growth and nutrient acquisition. Further studies are required to understand and explore the molecular mechanisms and signalling pathways associated with stress amelioration and crop development.

## Materials and methods

### Study area

The present study was performed in clay pot (~ 8 kg soil from the experimental plot) in the month of September, 2024 located in the Botanical Garden of BHU, Varanasi. The Located site lies in the Eastern Indo-Gangetic plains of India located at 25º 14' N, 82º 3' E and 76.1 m above MSL. Soil profile of the study pot was pale brown, alluvial and sandy loam with pH 7.3, collected from the experimental plot. Table 2 provides the meteorological variables of the study site during the experimental study.

### Plant materials

Two high yielding variety of Proso (cultivar TNAU 145 and TNAU 202) millet seeds were obtained from TNAU, India and stored in cool and dried conditions until start of the experiment. TNAU 202 and TNAU 145 were developed at the Department of Millets, TNAU university, Coimbatore and released in the year 2011 and 2007, respectively. TNAU 202 is rust resistant and brown spot diseases and tolerant of shoot fly. Conversely, TNAU 145 is resistant to rust. Proso millet TNAU 202 is the derivative of cross between PV 1453 x GPUP 16 and TNAU 145 is the cross over product of PV 1454 x TNAU 96. On an average, TNAU 202 (70–75 maturity days) and TNAU 145 (70–72 Maturity days) produces 18–20 (Q/ha) during the Kharif season. In TNAU 202, the panicle usually bears higher number of grains and are loosely branched whereas the panicle of TNAU 145 is large and more branched. Both the test cultivars are commonly cultivated in Uttar Pradesh, Bihar, Odisha, Madhya Pradesh, Uttarakhand, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, etc. states of India.

### *Experimental set up*

The experiment followed a complete randomized block design, having a pot set up under natural field conditions. A total of 12 pots were prepared in triplicates for each cultivar per treatment. The experimental pots were provided with ambient (A; 5.8 kJ $m^{-2}$ $day^{-1}$ biologically effective UV-B) and elevated UV-B (eUV-B; ambient + 7.2 kJ $m^{-2}$ $day^{-1}$) treatments. Q panel UV-B 313 40 W fluorescent lamps (Q panel Inc. Cleveland, OH, USA) was used for artificial treatment of eUV-B. Using adjustable steel frame, three lamps of 120 cm long were suspended 45 cm apart in perpendicularly above the top of each clay pot sown with two different Proso cultivar seeds (cultivar TNAU 145 and TNAU 202) placed at the experimental plot. 50 seeds of each Proso millet cultivar were sown in equidistant (4 cm deep inside the soil surface) in the tagged clay pots (levelled as 145 A for TNAU 145 ambient; 145 eUV-B for TNAU 145 eUV-B and 202 A for TNAU 202 ambient; 202 eUV-B for TNAU 202 eUV-B) and data for germination values (germination percentage and germination velocity) were retrieved. 10 seedlings were maintained for further analysis. The lamps for ambient (A) dose were enclosed with polyster filter (0.13 mm) to absorb radiation below 320 nm, while elevated UV-B (eUV-B) lamps are wrapped with thick cellulose diacetate filter (0.13 mm) to cut transmission down to 280 nm. The filters were replaced after 7 days to so that optical properties of the setup were maintained. The set-up provides eUV-B to plant canopy for 3 h (10:00 to 13:00 h) up to the seedling stage (till 15 DAG). UV meter (UVP Inc. San Gabriel, (A), USA) was used to measure the UV-B at plant canopy. Ambient (A) received 5.8 kJ $m^{-2}$ $day^{-1}$ and elevated (eUV-B) received ambient + 7.2 kJ $m^{-2}$ $day^{-1}$ biologically effective UV-B, thus mimicking 20% depletion of stratospheric ozone layer under clear sky conditions at Varanasi[90]normalized at 300 nm. Biologically effective UV-B doses were determined via comparing the values with spectro-power meter (Scientech, Boulder, USA) as per[91]. Manual weeding along with proper moisture regime was maintained during the entire duration of study.

## Plant sampling

Plants seedlings were sampled randomly in three replicates after two weeks of germination (DAG) for each cultivar per treatment. After samplings, plant samples were dipped and then homogenized in liquid $N_2$ and stored at −20 °C for further analysis.

### *Germination values*

GP (germination percentage) was calculated as per[92]in which number of germinated seeds (A) and total number of seeds (B) were manually counted.

Total germination percentage= (A/B) × 100.

GV (Germination velocity) was calculated by using an index given by[93]. Following is the formula for calculation of GV:

$$GV = [n_1/1 + n_2/2 + n_3/3 + .... + n_n/n] \times\ 100/1$$

where $n_1$, $n_2$, $n_3$, …$n_n$ are the percentage of seeds that were germinated on days 1, 2, 3, … n respectively.

### *Morphological measurements*

Shoot length (SL) and root length (RL) were measure by taking three plants in replicate for both the cultivar from both the treatments. For total biomass (TB) seedlings were uprooted with roots intact and washed carefully to eliminate all soil debris, kept in oven (80 °C) until dried.

### *Pigments*

Chlorophyll and carotenoids were measured as per the methodology of[94,95]. Anthocyanin concentration was calculated following the[96] in which 0.1 g leaf was homogenized in methanol containing $CaCO_3$ and HCl few drops. The readings were taken at 535 and 650 nm, respectively.

$$chlorphyll\ a\ (mg\ g^{-1}FW) = [(12.3 \times\ OD_{663} - 0.86 \times\ OD_{645}) \times\ V]/1000 \times\ w \times\ d$$

$$chlorphyll\ b\ (mg\ g^{-1}FW) = [(19.6 \times\ OD_{645} - 3.6 \times\ OD_{663}) \times\ V]/1000 \times\ w \times\ d$$

$$Total\ chlorphyll = chlorphyll\ a + chlorphyll\ b$$

$$Carotenoids\ \left(mgg^{-1}\ FW\right) = [(7.6 \times\ OD_{480} - 1.49 \times\ OD_{510}) \times\ V]\,/1000 \times\ w \times\ d$$

Where, V is volume of the extract, d is length of the light path and w is leaf tissue weight.

### *Bioactive components*

Fresh fully expanded leaf was taken for the determination of flavonoid and total phenols. Total phenols and flavonoid contents were determined as per protocol of[97,98] respectively.

### Physiological parameters

The maximum photosynthetic efficiency ($F_v/F_m$) was obtained by using a chlorophyll fluorescence meter (Handy PEA+, Hansatech Instruments Ltd., Norflok, UK). Prior to measurement, leaves were dark adapted for 30 min during morning on cloud-free days. $F_0$ refers to initial fluoroscence, $F_v$ variable fluorescence, and $F_m$ maximum fluoroscence and $F_v/F_m$ is photosynthetic efficiency were measured. OJIP fluorescence transients indicated O step (at 20µs) when all the PSII reaction centers are open, J step (at 2ms) intensity and I step (at 30ms) intensity and finally P step= (maximum fluorescence intensity) when all the PSII reaction centers are closed.

### ROS, solute leakage and lipid peroxidation

Protocol of[99] was followed for the estimation of Hydrogen peroxide ($H_2O_2$) content[100]. methodology was followed for determination of $^{\bullet}O_2^-$ production rate. Solute leakage determined by following the method of[101] using EC (conductivity) meter (Model- PCTestr 35)[102]. methodology was followed for measurement of lipid peroxidation.

### Antioxidative defense mechanism

Determination of enzymatic antioxidants, protocol of[103] was followed. Phosphate buffer solution containing polyvinylpyrrolidone (PVP), Triton-X 100, EDTA, served as the extraction medium for assessing total protein content and enzymatic antioxidants. Leaf samples were crushed in extraction buffer, with the addition of 100 µl of PMSF during each homogenization step. Following homogenization, the samples were subjected to centrifugation at 4 °C. The resulting supernatant was stored in deep freezer for analysing enzymatic antioxidants (SOD, CAT and APX) activity and protein content.

### Nutritional quality

Nutritional quality of the germinated seedling was assessed in which total soluble sugars (TSS), reducing sugar (RS) and starch content (SC) were measured using the protocol of[104]. Total free amino acids (TFAA) were quantified by[105,106]. methodology was followed for quantification of protein content.

### Statistical analysis

Statistical analysis was done by using SPSS (SPSS Inc., Version 22). Mean and standard error were calculated for both the treatments. Paired sample t-test was used to analyze significance difference between both the treatments. Two-way ANOVA was done to check the significance due to treatment (T) and Cultivar (Cv). Graphs were produced using GraphPad Prism 8.0.1 software (https://www.graphpad.com/features).

## Data availability

All the data generated or analysed during this study are included in this article.

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## Author contributions

Rikina Choudhury: Data curation, analysis and interpretation, Writing original draft. Pooja Singh: Data analysis, Interpretation, review, and editing. Krishna Kumar Choudhary: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Validation, Visualization, Writing & editing. All authors read and approved the final manuscript.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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