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## Ultraviolet-C irradiation of wheat grains induces seedling resistance to leaf rust and powdery mildew disease

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### Highlights

- Grain treatment with UV-C improved wheat seedling resistance to leaf rust and powdery mildew disease.
- The most reduction in the disease parameter was detected in seedlings produced from UV-C treated germinated grains.
- Disease severity was significantly reduced in response to UV-C for 10 minutes by up to 68 and 63% for leaf rust and powdery mildew disease, respectively.
- UV-C radiation induces resistance in plants by promoting the accumulation of phenolics, oxidative enzymes, and total chlorophyll, in addition to the modification of phenylalanine ammonia-lyase mRNA expression levels.

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Key words: wheat, leaf rust, powdery mildew, UV, phenolic compounds, enzyme activity, RT-PCR.

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### Abstract

Ultraviolet-C (UV-C) irradiation of grains activated the antioxidant system and wheat seedlings' resistance to leaf rust and powdery mildew disease under greenhouse conditions. Two wheat cultivars (Gemmeiza-12 and Sids-1) with dry and germinated grains were treated with UV-C at three exposure times (5, 10, and 15 minutes). The results indicated that the percentages of disease severity and infection type for leaf rust and powdery mildew on wheat seedlings were significantly reduced when exposed to UV-C at all exposure times compared to the untreated control. The most effective treatments for both cultivars were obtained in seedlings grown from germinated grains treated with UV-C for 10 minutes. Furthermore, UV-C irradiation treatments improved plant resistance to infection by activating certain defense genes, thereby increasing the production of resistance compounds that support defense mechanisms against pathogens. Our results demonstrated that UV-C for 10 minutes can induce resistance in wheat seedlings while also increasing total chlorophyll, total phenolic compounds, phenylalanine ammonia-lyase, and peroxidase activity. In addition, phenylalanine ammonia-lyase mRNA expression levels were significantly increased in seedlings growing from germinated grains treated with UV-C for 10 minutes, as compared to both infected and uninfected controls. These findings demonstrate the potential for additional UV-C radiation treatments to enhance disease resistance.

### Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important cereals, providing calories and protein to approximately 85% of the global population (Ma *et al.*, 2014; Zhang *et al.*, 2017). Among the most significant plant diseases are leaf rust and powdery mildew, which cause substantial yield losses in wheat (Zhang *et al.*, 2016; Najeeb *et al.*, 2019; Arab *et al.*, 2021). *Blumeria graminis* DC E.O. Speer f. sp. *tritici* Em. Marchal (*Bgt*) syn. *Erysiphe graminis* DC causes powdery mildew (Gao *et al.*, 2018).

Once wheat is severely infected with powdery mildew, grain yield losses can reach 40% (Li *et al.*, 2011). Under normal Egyptian growth conditions, *B. graminis* f. sp. *tritici* affects the majority of common wheat cultivars, resulting in a grain yield reduction of 26.68% in highly susceptible cultivars (Draz *et al.*, 2019). Wheat is susceptible to a common and widespread foliar disease caused by *Puccinia triticina* Eriks (Huerta-Espino *et al.*, 2011). Early leaf rust infection causes significant yield losses of up to 30% (Khan *et al.*, 2013; Atia *et al.*, 2021). Some studies have documented up to 60% yield reductions in highly susceptible wheat cultivars (Smith, 2008; El-Orabey *et al.*, 2017; Arab *et al.*, 2021). In Egypt, yield losses of wheat due to leaf rust have reached up to 50% (Thabet and Najeeb, 2017). The development of resistant varieties is considered the most practical, efficient, and sustainable method to manage these diseases (El-Shamy *et al.*, 2016). However, chemical fungicides have been the standard method for managing wheat diseases. Nevertheless, their limitations necessitate the development of new methods, particularly for protective culture and organic agriculture. This century has witnessed an expansion of research into alternative physical techniques known as emerging technologies. In this manner, various physical methods are applied at various stages of plant growth, and the most effective, cost-effective, and environmentally safe method is to effectively control the diseases (Govindaraj *et al.*, 2017). Pre-sowing physical techniques aim to increase crop yield by accelerating plant reaction speed to biotic and abiotic factors during and after germination (Thomas and Pothur 2017; Mariz-Ponte *et al.*, 2018). The induction of resistance mechanisms by pre-sowing physical technique is similar to creating a stress memory in plants, which is associated with chromatin alterations, transcription elements, phytohormones, and stress-regulating metabolites (Conrath, 2011). Ultraviolet (UV) radiation has emerged as a promising technique for disease prevention among these physical methods (Araujo *et al.*, 2016; Rifna *et al.*, 2019).

UV radiation has traditionally been divided into three wavelength ranges: UV-A (320-390 nm), UV-B (280-320 nm), and UV-C (100-280 nm). Within these ranges, excess levels of UV-C radiation are both photochemically and biologically lethal. UV radiation is a significant factor in disrupting the normal biological functions of all organisms by causing DNA damage. Low-dose, non-detrimental UV-C exposures induce beneficial responses in the treated organism. Numerous studies have described how UV-C radiation induces resistance in plants by promoting the accumulation of flavonoids, phenolics, oxidative enzymes, and phytoalexins, in addition to the modification of cell walls and cell death (Turtoi, 2013; Urban *et al.*, 2016; Vanhaelewyn *et al.*, 2020). Furthermore, UV-C irradiation has been shown to stimulate the germination and growth parameters of maize and wheat grains (Rupiasih and Vidyasagar, 2016; Sukthavornthum *et al.*, 2018; Sadeghianfar *et al.*, 2019; Korotkova *et al.*, 2020). Biochemical antioxidant enzymes like peroxidases and polyphenol oxidase have been shown to mitigate photo-oxidative stress by reducing reactive oxygen species (ROS) in plants. It is also considered a critical defensive component against pathogenic attacks (Zu *et al.*, 2011). Furthermore, UV-C treatments applied to seeds have been demon-

strated to increase their resistance to pathogen attack (Brown *et al.*, 2001; Scott *et al.*, 2019; Aboul Fotouh *et al.*, 2019). However, no study has examined the impact of UV-C grain treatment radiation on the induction of disease resistance in wheat plants to fungal foliar diseases. Consequently, this research aimed to understand the effect of different durations of irradiation of wheat grain with UV-C on plant physiological and biochemical markers in wheat seedlings, which can enhance the resistance to powdery mildew and leaf rust disease under greenhouse conditions.

## Materials and Methods

### Plant and fungal

Gemmeiza-12 (highly susceptible to powdery mildew disease) and Sids-1 (highly susceptible to leaf rust disease) are two Egyptian wheat cultivars provided by the Wheat Research Section, Field Crop Institute, Agriculture Research Center (ARC), Giza, Egypt (Table 1).

The inoculum source for *B. graminis* f.sp. *tritici* (PGT) was obtained from field-grown wheat plants naturally infected with powdery mildew. The uredia of *P. triticina* were kindly provided by the Wheat Diseases Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt. The following experiments were carried out in the greenhouses and laboratories of the Department of Plant Pathology, Faculty of Agriculture, Ain Shams University.

### Ultraviolet-C irradiation grain treatment

The wheat grains of each cultivar were first disinfected with 1% NaOH for 5 minutes, then washed 3-4 times with sterilized distilled water. The grains were divided into two groups; the first group was immediately treated with UV-C. The other group was soaked in distilled water for 24 hours and then arranged on a plate coated with two layers of cotton. Grains were cultivated for two days in an incubator at 25°C until they germinated, then treated with UV-C. Dry and germinated grains were irradiated with UV-C at three exposure times (5, 10, and 15 min) using a UV-C fluorescent lamp (TUV 15 W G158T8, wavelength 254 nm, Phillips, Germany). In accordance with Brown *et al.* (2001), the targeted irradiation surface for plates was at a 20 cm distance from the lamp. After UV-C treatment, grains were placed in complete darkness all day (24 hours) to inhibit photoreaction activities. As controls, grains that were not exposed to UV-C were maintained. The treated grains (dry and germinated) were then planted at a rate of 5 grains/plastic pot (15 cm) containing clay soil. The experiment was conducted in a split-plot arrangement in a randomized complete design with five replicates in the greenhouse. After seven days, seedlings were inoculated with powdery mildew and leaf rust disease.

**Table 1.** Gemmeiza-12 and Sids-1 cultivars pedigree.

Genotypes	Pedigree	Year of release
Gemmeiza-12	OTUS/3/SARA/THB//VEECMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM	2011
Sids-1	HD2172/Pavon "S"//1158.57/Maya74 "S" SD46-4Sd-2SD-1SD-0SD	1996

### Inoculation and assessment of leaf rust disease

The Sids-1 cultivar seedlings were inoculated with uredia of *P. triticina*, as described by Tervet and Cassel (1951). After inoculation, seedlings were incubated in dark, moist chambers for 24 hours, transferred to their designated greenhouse benches, and kept under observation for 15 days. Five pots without infection with the pathogen and untreated with UV-C served as controls (healthy plants), and disease severity was assessed 15 days after inoculation. Assessment of leaf rust was based on the symptoms observed on the entire plant of each replicate, which were classified as resistant or susceptible based on the infection types corresponding to Stakman *et al.* (1962), as shown in Table 2, and disease severity (%) was recorded according to Long *et al.* (1994).

### Inoculation and assessment of powdery mildew disease

The Gemmeiza-12 cultivar seedlings were inoculated with *B. garminis* f.sp. *tritici* by shaking conidia from naturally infected plants collected from commercial wheat fields in several areas across Egypt. After inoculation, seedlings were kept in complete darkness at a relative humidity of 98% for 24 hours (El-Shamy *et al.*, 2012). The inoculated seedlings were maintained in the greenhouse at 25°C, 70-90% relative humidity, and kept under observation until evaluation. Five pots without infection with the pathogen and untreated with UV-C served as controls (healthy plants), and disease severity was assessed 15 days after inoculation. Disease severity of powdery mildew was measured by estimating the percentage of leaf area infected on the whole plant of each replicate using the modified Cobb scale of 0 to 100% (Peterson *et al.*, 1948). Infection types were scored using a modified 0-to-4 scale (Wang *et al.*, 2005) for recording the host response to infection, as depicted in Table 3.

### Determination of leaf chlorophyll

Leaf chlorophyll content was measured 15 days after inoculation using a hand-held portable optical meter (Minolta SPAD-502 Plus chlorophyll meter, Tokyo, Japan). Soil plant analysis development (SPAD) measurements were performed on ten plants per treatment (Novichonok *et al.*, 2016). Afterward, chlorophyll *a* was determined by converting the SPAD values to mg/m<sup>2</sup> in accordance with Monje and Bugbee (1992) using Equation 1:

$$\text{Chlorophyll } (a) = 80.05 + 10.4 \times \text{SPAD value} \quad (1)$$

**Table 2.** Infection types of wheat leaf rust.

Host	Infection type	Disease symptoms
Resistant	0	No uredia or other macroscopic signs of infection
	0	No uredia but hypersensitive necrotic or chlorotic flecks present
	1	Small uredia surrounded by necrosis
	2	Small to medium uredia surrounded by chlorosis or necrosis
Susceptible	3	Medium-sized uredia that may be associated with chlorosis
	4	Large uredia without chlorosis or necrosis or rarely necrosis

**Table 3.** Infection types of wheat powdery mildew.

Host	Infection type	Disease symptoms
Resistant	0	No visible symptoms
	0	Hypersensitive necrotic flecks
	1	Minute colonies with few conidia produced
	2	Colonies with moderately developed hyphae
Susceptible	3	Colonies with well-developed hyphae and abundant conidia, but colonies not joined together
	4	Colonies with well-developed hyphae and abundant conidia, and colonies mostly joined together

### Assay of total phenolic compounds

Fifteen days after inoculation, 1 g of fresh wheat leaf samples were used to extract total phenolic compounds using ethanol according to the method presented by Swain and Hillis (1955). According to Vlase *et al.* (2014), the assay of the total phenols was carried out. A spectrophotometer (Unico-2100, Dayton, NJ, USA) was used to assess the optical density of the developed blue color at 725 nm. A catechol standard curve was utilized to measure the total quantity of phenols.

### Extraction and assay of antioxidant enzyme activity

Five replicates of fresh leaf samples were collected from seedlings 15 days after inoculation, ground in liquid nitrogen, and then frozen at -80°C for further biochemical analysis. The techniques described by Biles and Martyn (1993) were primarily utilized to isolate peroxidase, as follows: 1 g of leaf tissue was ground in sodium phosphate buffer (2 mL, 0.1 M, pH 6.5). Samples were centrifuged for 20 minutes at 12000 rpm at 4°C. Peroxidase activity was estimated according to Liu *et al.* (2010) with 2.9 ml of sodium phosphate buffer (100 mM, pH 6.0) including 0.25% (v/v) guaiacol and hydrogen peroxide (100 mM). First, 100 µl of the crude enzyme extract was added to initiate the reaction. At 470 nm, absorbance variations were measured using a Unico UV-2100 spectrophotometer (Unico-2100, Dayton, NJ, USA). An increase in absorbance min<sup>-1</sup>/g<sup>-1</sup> of fresh weight represented the enzyme activity. Phenylalanine ammonia-lyase (PAL) activity was defined following the procedure of Solecka and Kacperska (2003) as follows: 1 g of leaf tissue was extracted in borate buffer (2 mL, 50 mM, PH 8.8). The extracts were centrifuged at 12000 rpm for 10 minutes at 4°C, then 1 mL of the supernatant was blended with sodium borate buffer (2 mL, pH 8.8) and 1 mL of 10<sup>-2</sup> M L-phenylalanine. After 1 hour of incubation at 30°C, the reaction was stopped by inserting 500 µl of HCl (6N). The mixture was then centrifuged at 12000 rpm for 10 minutes. Enzyme activity was conveyed as trans-cinnamic acid formed using a Unico UV-2100 spectrophotometer (Unico-2100, Dayton, NJ, USA) at 290 nm.

### RNA expression of phenylalanine ammonia-lyase

Reverse transcription polymerase chain reaction (RT-PCR) was used to assess the effect of UV light treatments on the expression of PAL. Using a total RNA purification kit (Bioscience,

Dümmer, Germany), total RNA was extracted from the leaves of Sids-1 and Gemmeiza-12 seedlings obtained from irradiated germinated grains 15 days after inoculation. Subsequently, mRNA was reverse transcribed into cDNA using a one-step RT-PCR kit (QIAGEN, Hilden, Germany). The sequence of the forward wheat PAL primer is (PAL-F, 5- A A G C T G A T G T T C G C G C A G T T C T - 3), and the reverse primer is (PAL-R, 5- A A A C C A T A G T C C A A G C T C G G T - 3). The expression of the target gene was compared relative to the housekeeping wheat actin genes (actin-F, 5-C T C A T A C G G T C A G C A A T A C -3; actin-R, 5-A T G T G G A T A T C A G G A A G G A -3). In a thermal Eppendorf master cyclor (T100TM thermal cyclor, BIO-RAD, Segrate, Italy). This PCR reaction sequence consists of the following steps: the first step is reverse transcription at 50°C for 30 minutes, then denaturation at 94°C for 15 minutes, 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds, and the last step is the final extension at 72°C for 1 minute. On 1.5% agarose gels stained with ethidium bromide, the amplification products were visualized and photographed using a gel documentation system (Bio-Doc Analyze, Biometra, Göttingen, Germany).

### Statistical analysis

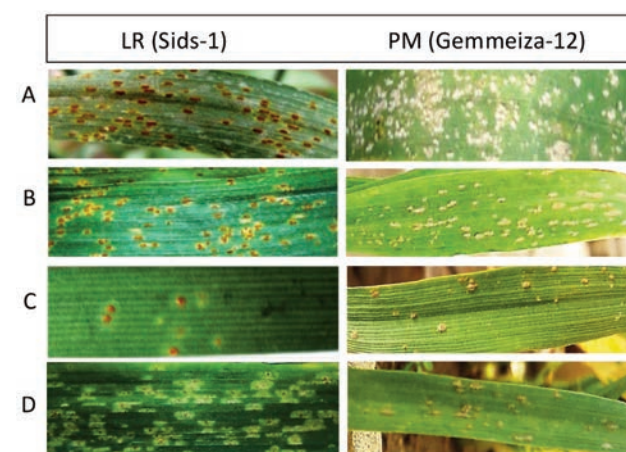
Data were statistically analyzed using analysis of variance using SAS software (Cary, NC, USA). Duncan's multiple range test was utilized to identify homogenous groups of means that differ significantly at 5% ( $p \leq 0.05$ ) (Duncan, 1955).

## Results

### Effect of ultraviolet-C radiation on leaf rust and powdery mildew disease

As shown in Table 4, UV-C irradiation at all tested exposure times was associated with a reduction in disease severity and infection type in seedlings germinated from treated grains compared to those germinated from untreated grains. In addition, the degree of disease parameter reduction in both cultivars depended on the duration of UV-C exposure and the grain application form. The most effective treatment in both cultivars was obtained from seedlings grown from germinated grains treated with UV-C for 10 minutes. Data presented in Table 4 and Figure 1 demonstrate that treatment of Sids-1 germinated grains with UV-C for 10 minutes

significantly reduced the severity of leaf rust disease to around 25% as compared to the infected control treatment (67%). In addition, there was a positive correlation between UV exposure times and infection type. The lowest infection type (1 and 0;) was obtained on seedlings grown from germinated grain treated with UV-C for 10 minutes, respectively, compared with the infected control treatment (4). The same decreasing trend was also observed for the Gemmeiza-12 cultivar. Treatment of germinated grains with UV-C for 10 minutes decreased the severity of powdery mildew disease to around 19% compared to the infected control treatment (60%). The lowest infection types (1 and 2) were obtained for seedlings grown from germinated grain treated with UV-C for 10 minutes, compared with the infected control treatment (4). Compared to the infected controls, disease severity was significantly ( $p \leq 0.05$ ) reduced in response to UV-C for 10 minutes by up to 68 and 63% for leaf rust and powdery mildew disease, respectively.



**Figure 1.** Effect of UV- C grain irradiation treatments on infection type and severity of leaf rust (LR) caused by *Puccinia triticina* and Powdery mildew (PM) caused by *Blumeria graminis* f.sp *tritici* in seedlings of Sids-1 and Gemmeiza-12 wheat cultivars at 15 days post-inoculation under greenhouse conditions. wheat grains were irradiated with UV-C at three exposure times (5, 10 and 15 min) which A: control, B: UV- 5 min, C: UV- 10 min, D: UV- 15 min.

**Table 4.** Effect of UV-C grain irradiation treatments on infection type and severity of leaf rust and powdery mildew disease in Sids-1 and Gemmeiza-12 cultivars at 15 days post-inoculation under greenhouse conditions.

Treatment	Sids - 1 (Leaf rust disease)				Gemmeiza-12 (Powdery mildew disease)			
	Germinated		Dry		Germinated		Dry	
	IT	DS %	IT	DS%	IT	DS%	IT	DS%
Control	4	67 <sup>a</sup> ± 1.45	4	78 <sup>a</sup> ± 1.66	4	60 <sup>c</sup> ± 2.60	4	72 <sup>b</sup> ± 1.66
UV-5m	2 & 3	46 <sup>d</sup> ± 1.85	3	55 <sup>c</sup> ± 2.88	3	39 <sup>ef</sup> ± 2.08	3 & 4	45 <sup>d</sup> ± 2.88
UV-10m	0; & 1	25 <sup>j</sup> ± 2.88	1 & 2	34 <sup>gh</sup> ± 2.33	1 & 2	19 <sup>k</sup> ± 1.73	2	29 <sup>hij</sup> ± 2.08
UV-15m	2	33 <sup>ghi</sup> ± 1.45	2	44 <sup>de</sup> ± 2.33	2	27 <sup>ij</sup> ± 1.45	3	37 <sup>fg</sup> ± 1.66
LSD at 5%	--	5.44	--	5.44	--	5.44	--	5.44

†All the statistical differences were presented relative to the untreated control. Data are means of three replicates ± SE; means with different letters within columns are significantly different by Duncan's multiple range test at ( $p \leq 0.05$ ). DS: Disease severity. IT: Infection type, UV- 5/10/15 min: wheat grains were irradiated with UV-C at three exposure times (5, 10 and 15 minute).

### Physiological parameters and induction of resistance with ultraviolet-C radiation

To assess the induction of resistance induced by UV-C grain irradiation treatments in wheat seedlings infected with leaf rust and powdery mildew, the total of chlorophyll (SPAD value), chlorophyll *a*, and phenol compounds, as well as peroxidase and PAL enzyme activities, was measured.

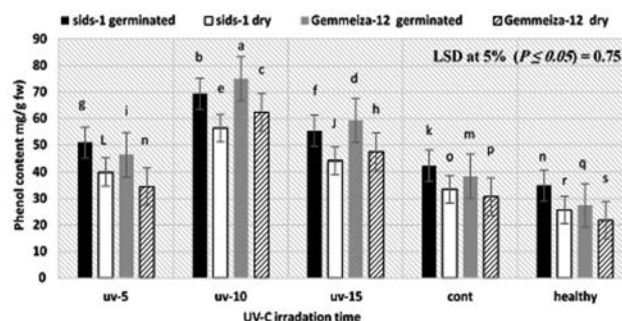
#### Chlorophyll content

The maximum increase in SPAD value and chlorophyll *a* was observed in the leaves of non-infected seedlings grown from untreated grains (healthy controls), as shown in Figure 2. Moreover, our findings demonstrated that the concentrations of total chlorophyll and chlorophyll *a* in seedlings grown from UV-C pretreated grains at all exposure times were significantly higher compared to seedlings grown from untreated grains (infected controls) in both cultivars, Sids-1 and Gemmeiza-12, respectively. When germinated grains were irradiated with UV-C for 10 minutes, the total chlorophyll content of Sids-1 seedlings increased by 23.6% compared to infected control plants. During this exposure period, the chlorophyll concentration increased to 326.2 mg/m<sup>2</sup> compared to infected control plants. Similar effects were observed in Gemmeiza-12 seedlings, where the higher increases in the total chlorophyll concentration were 24 (SPAD value), and chlorophyll *a* increased with values of 330.3 mg/m<sup>2</sup> relative to infected control plants.

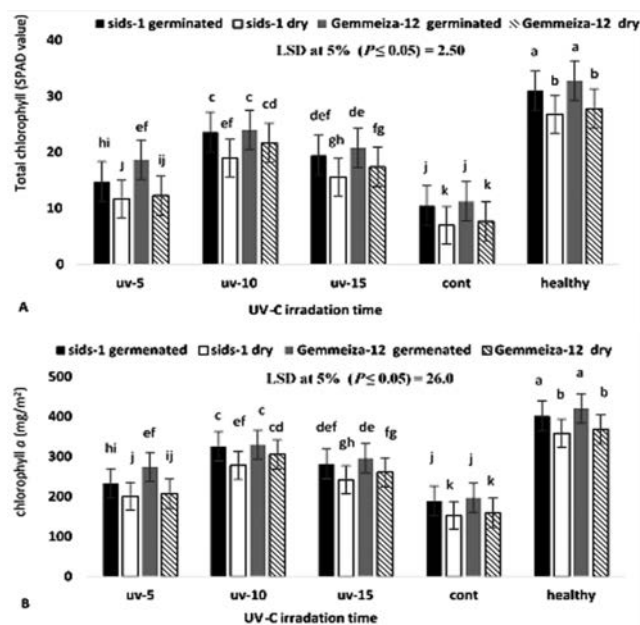
#### Enzyme and total phenolic compound activities

Total phenolic compounds significantly increased under all UV-C treatments in both cultivars (Figure 3). Values ranged from

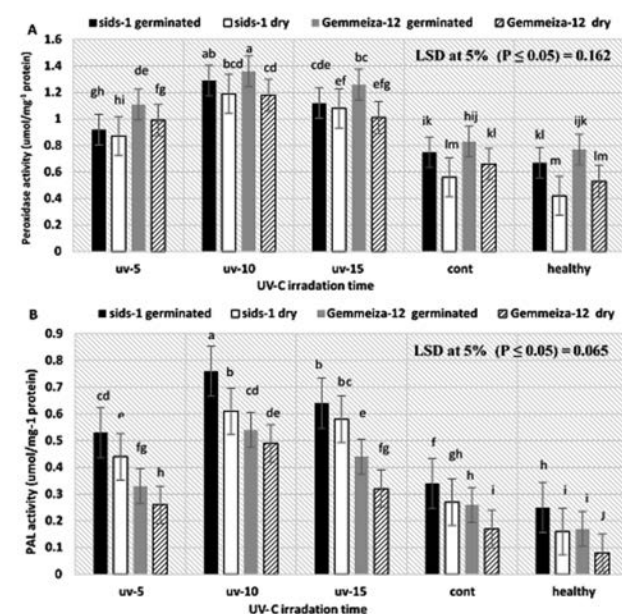
30 to 42 mg/g fw in the infected control. Under UV-C treatment for 10 minutes, they were increased to 69.4 in Sids-1 and 75 mg/g fw in Gemmeiza-12 seedlings derived from irradiated germinated grains, respectively. Furthermore, our results revealed a significant positive effect on antioxidant enzyme (resistance-related enzymes) activity under UV-C treatments (Figure 4). Germinated grains treated with UV-C for 10 minutes in both cultivars revealed a higher effect on the activation of the peroxidase and PAL enzymes in wheat seedlings as compared with the controls.



**Figure 3.** Effect of UV-C grain irradiation treatments on phenol content mg/g fw in seedlings of Sids-1 and Gemmeiza-12 wheat cultivars at 15 days post-inoculation under greenhouse conditions. wheat grains were irradiated with UV-C at three exposure times (5, 10 and 15 min). cont., plant infected/untreated with UV; healthy, plant uninfected/untreated with UV. Error bars, standard error.



**Figure 2.** Effect of UV-C grain irradiation treatments on total chlorophyll (A) and chlorophyll *a* content (B) in seedlings of Sids-1 and Gemmeiza-12 wheat cultivars at 15 days post-inoculation under greenhouse conditions. wheat grains were irradiated with UV-C at three exposure times (5, 10 and 15 min). cont., plant infected/untreated with UV; healthy, plant uninfected/untreated with UV. Error bars, standard error.



**Figure 4.** Effect of UV-C grain irradiation treatments on (A) Peroxidase and (B) PAL enzymes activity in seedlings of Sids-1 and Gemmeiza-12 wheat cultivars at 15 days post-inoculation under greenhouse conditions. wheat grains were irradiated with UV-C at three exposure times (5, 10 and 15 min). cont., plant infected/untreated with UV; healthy, plant uninfected/untreated with UV. Error bars, standard error.

### RNA expression of phenylalanine ammonia-lyase

PAL genes were efficiently transcribed into mRNA, as evidenced by the presence of specific amplicons of expected molecular weight (104 bp) in both cultivars (Figure 5 A and B). However, the expression pattern of the PAL gene varied in the semi-quantitative RT-PCR analysis. The expression of PAL genes does not change in the 5-minute treatment compared to the infected, untreated control. The highest gene expression of the enzyme is recorded in the seedlings grown from germinated grains and treated with UV-C for 10 minutes in both cultivars, Sids-1 and Gemmeiza-12, infected with leaf rust and powdery mildew disease, respectively. As an internal control for cDNA synthesis, specific actin transcript amplicons were detected in all plants (Figure 5C).

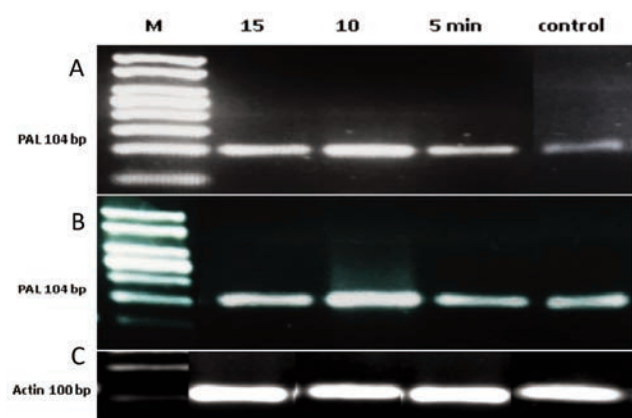
### Discussion

UV irradiation has gained popularity in recent years since it promotes plant growth and increases resistance to biotic and abiotic stresses, thereby increasing crop yield. The pre-sowing treatment of seeds with ultraviolet radiation has received particular attention (Windram *et al.*, 2012; Thomas and Puthur, 2017; Mariz-Ponte *et al.*, 2018). Numerous studies have demonstrated that UV-C has a beneficial effect on the physiological and biochemical processes in seeds and plants, as well as on the health of seeds, germination, and seedling strength of a variety of crops (Rupiasih and Vidyasagar, 2016; Castronuovo *et al.*, 2017; Sadeghianfar *et al.*, 2019; Semenov *et al.*, 2020; Hernandez-Aguilar *et al.*, 2021). In the current study, UV-C irradiation of wheat grains (dry and germinated) at three exposure times (5, 10, and 15 minutes) increased resistance to leaf rust and powdery mildew disease. Similar patterns were observed in treated seedlings, which showed a significantly reduced severity and infection type of the pathogen compared with the infected, untreated control treatment. In both cultivars, the most effective treatments were obtained in seedlings grown from germinated grains treated with UV-C for 10 minutes,

followed by UV-C for 15 minutes. It is suggested that the reaction was only caused by induced resistance because the pathogen was not directly exposed to UV-C light. The generation of wheat resistance *via* UV-C grain treatment matched the findings of Scott *et al.* (2019), which indicated that treatment of seeds with UV-C radiation had a positive effect on the decrease in disease incidence in tomato plants caused by *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *Lycopersici* and *Sclerotinia minor*. According to Falconi and Mendizabal (2018), the treatment of lupin seeds with UV-C significantly reduces seedborne infections of anthracnose caused by *Colletotrichum acutatum*. Furthermore, Aboul Fotouh *et al.* (2019) and Siddiqui *et al.* (2011) illustrated that irradiation of germinated green bean, mung bean, and groundnut seeds with UV-C for 60 minutes decreased infection by root-infecting fungi, *i.e.*, *Rhizoctonia solani*, *Macrophomina phaseolina*, and *Fusarium* spp. Moreover, Brown *et al.* (2001) stated that exposure of cabbage seeds to UV-C decreased the density of *Xanthomonas campestris* pv. *campestris* in infected leaves. On the other hand, Aarouf and Urban (2020) indicated that exposure to UV-C light for one or 60 seconds increased plant resistance to *Botrytis cinerea*, *Phytophthora capsici*, and *Plasmopara viticola* in the leaves of lettuce, pepper, tomato, and grapevine plants. In contrast, Wang *et al.* (2018) explained that when UV-B radiation was used only prior to *Puccinia striiformis* f. sp. *Tritici* inoculation, the incubation period was shortened, and the infection efficiency, sporulation quantity, and disease index increased. All these effects of pre-treatment of seeds with UV-C radiation are related to two distinct processes: the direct germicidal activity of UV-C on pathogenic surface microbes and the stimulation of host resistance as a result of UV-induced stress reactions in treated tissues (Semenov *et al.*, 2017).

Induction of resistance in plants with UV radiation has been related to the activation of different host defenses, including the accumulation of phenolic and flavonoid compounds combined with the stimulation of antioxidant mechanisms and delayed chlorophyll degradation (Sharma *et al.*, 2012; Shin *et al.*, 2013; Urban *et al.*, 2016; Xu *et al.*, 2019; Vanhaelewyn *et al.*, 2020; Elshafei *et al.*, 2021). The totals of chlorophyll, chlorophyll *a*, and phenol compounds, as well as peroxidase and PAL enzyme activity, were measured to evaluate the role of UV-C grain irradiation in inducing resistance in wheat seedlings. The current study found that different UV-C treatment exposure times significantly increased the aforementioned parameters compared to their corresponding controls. In both cultivars that were UV treated, a higher increase was obtained in seedlings grown from germinated grains that were UV-C treated for 10 minutes, followed by UV-C for 15 minutes. The increase in leaf total chlorophyll content corresponds to the results of other authors (Kacharava *et al.*, 2009; Siddiqui *et al.*, 2011; Sztatelman *et al.*, 2015; Falconi and Mendizabal, 2018; Semenov *et al.*, 2021). Chlorophyll's insensitivity to UV radiation may be linked to the activation of carotenogenesis or the stimulation of anthocyanidin synthesis. These latter compounds offer adequate defense against free radicals (Rice-Evans *et al.*, 1997). In contrast, Middleton and Teramura (1993) found that UV radiation stimulated the biosynthesis of UV-absorbing compounds and carotenoids, thereby achieving a photoprotective function.

Based on the aforementioned findings, total phenolic compounds exhibited a significant increase under all UV-C treatments in both cultivars. Furthermore, our results demonstrated a significant positive effect on antioxidant enzyme activity under UV-C treatments. However, germinated grains treated with UV-C for 10 minutes in both cultivars showed a higher response to peroxidase and PAL activation in wheat seedlings when compared to controls. These results are consistent with Kacharava *et al.* (2009) and



**Figure 5.** Effect of UV-C grain irradiation treatments on RNA expression of PAL gene in seedlings growing from germinated grains of (A) Sids-1 and (B) Gemmeiza-12 wheat cultivars at 15 days post-inoculation under greenhouse conditions. wheat grains were irradiated with UV-C at three exposure times (5, 10 and 15 min). RNA expression of PAL gene compared to actin gene expression (Housekeeping gene) (C). M: (marker), 100 bp DNA Ladder (Bio science).

Ouhibi *et al.* (2014), who illustrated that irradiation of kidney bean and lettuce seeds with low doses of UV-C enhanced the tocopherol, phenolic compounds, and flavonoids content in plant leaves. Similarly, McLay *et al.* (2020) concluded on the pre-treatment of lettuce seedlings with UV-B-induced phenolics, which act as phytoanticipins to restrict the growth of biotrophic pathogens (*Bremia lactucae*). Additionally, Falconi and Mendizabal (2018) demonstrated that seedlings grown from UV-C treated seeds revealed an enhanced concentration of the defense enzymes peroxidase and catalase compared to plants grown from untreated seeds. Similarly, Rivera-Pastrana *et al.* (2014) found a substantial increase in the activity of peroxidase and catalase on papaya peel as an effect of UV-C treatment compared to primary rates. Yongmei *et al.* (2018) revealed that the application of UV-B radiation before *M. oryzae* infection improved the activity of pathogenesis-related proteins like PAL, lipoxygenase, chitinase, and  $\beta$ -1,3-glucanase and the content of resistance-related elements, such as flavonoids and total phenols, thus enhancing the disease resistance of rice leaves to infection. It is well known that phenols play essential functions in plants as antifungal, antibacterial, and antiviral compounds (Gogoi *et al.*, 2001; Hammerschmidt, 2005). The antioxidant enzymes peroxidases, catalase, glutathione reductase, and superoxide dismutase help plants reduce oxidative stress. In addition, antioxidant enzymes can protect plant cells from photo-oxidative damage by scavenging ROS produced by UV-induced stress (Rao *et al.*, 1996). Peroxidase enzymes are associated with cross-linking cell wall components, lignin and suberin monomers polymerization, and consequent resistance in other host-pathogen relationships (Glazener, 1982). The discovery of a priming-related expression profile for PAL after inoculation suggests that gene priming may also be essential for facilitating a rapid response to the initial plant-pathogen interaction (Scott *et al.*, 2018). PAL is a crucial rate-limiting enzyme in phenylpropanoid compound metabolism. In addition, it regulates the biosynthesis of flavonoids, a compound believed to be responsible for the resistance of crop leaves (Chandrasekaran *et al.*, 2017; Li *et al.* 2018; Omar *et al.*, 2021). PAL is also involved in the synthesis of phytoalexins, which have potent antimicrobial properties and contribute to the structural reinforcement of the cell through lignification and the production of salicylic acid (Dixon *et al.*, 2002). In our study, RT-PCR data clearly revealed that the expression of the PAL gene was significantly upregulated in seedlings growing from germinated grains treated with UV-C radiation, as determined by RT-PCR. In agreement with our previous findings, the RNA and protein levels of the PAL gene are significantly upregulated in response to fungal infection, indicating its involvement in the host's immune response. These results agree with the findings of (Kobayashi *et al.*, 2013; Atia *et al.*, 2021) who demonstrated that exposure of rose plants to low levels of UV radiation and infection with powdery mildew disease induced the expression of PAL genes, which are implicated in secondary metabolic pathways. According to Yongmei *et al.* (2018), increased UV-B radiation prior to *Magnaporthe oryzae* infection significantly increased the expression of resistance-related genes (OsPAL and OsCHT), enhancing rice leaf disease resistance. Additionally, Pombo *et al.* (2010) showed that pre-storage UV-C treatment of strawberry fruit reduces *Botrytis cinerea* losses. After 4 and 24 hours of storage, the expression and enzyme activity of PAL increased compared to the level found in the control.

## Conclusions

According to the current study's findings, grain treatment with UV-C improved wheat seedling resistance to leaf rust and powdery mildew disease by activating the antioxidant system. The most significant decrease in the disease parameter was detected in seedlings produced from UV-C treated germinated grains. Therefore, it is possible that UV-C increased plant defense *via* multiple mechanisms, which requires further investigation at the level of resistance gene expression.

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