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Priming of Long-Term Stored Cotton Seeds Using Combined UV-A, B and C Radiation and Its Influence on Germination

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Seeds vigour and uniform germination across diverse environmental conditions is a primary objective in agriculture. Moisture and temperature are key factors affecting cotton seed quality during storage, where maintenance is rather difficult during long-term storage. To investigate the potential influence of UV radiation on enhancing cotton seed vigour after long-term storage, Deir Al-Zour 22 cotton variety was selected due to its reduced-vigour by time and its low germination rates. Germination rates of cotton seeds exposed to combined UV-A, B and C irradiation for different periods of time (4, 8, 12, and 16 min) were enhanced compared to non-irradiated seeds. Data showed improved growth of generated seedlings on PEG and NaCl supplemented media. Results showed no major changes on the expression of *GA3ox1* gene, whereas, two stress-related genes *DEH* and *VPP* were temporarily activated after treatment with UV-irradiation supporting their function as scavenging and accumulating factors of ROS, a typical by-product of the photo-excitation under UV. Our results suggest the possibility of using combined UV-A, B and C radiation as a physical priming agent of cotton seeds to induce plant vigor and enhance germination under stress conditions without affecting normal growth.

Key words: Ultraviolet; priming; cotton; gene expression; flow cytometry

The effects of ultraviolet (UV) radiation on terrestrial ecosystems and the agricultural sector have been extensively investigated due to the compelling issues such as climate change, thinning of the stratospheric ozone and enhanced level of solar ultraviolet-B radiation which can have serious impacts on global food security (Liu *et al.*, 2004; Van Dingenen *et al.*, 2009). The main three wavelength ranges of UV rays are UV-C (200-280 nm), UV-B (280-320 nm) and UV-A (320-400 nm). It was suggested that each 1 % reduction in ozone causes an increase of 1.3-1.8 % in UV-B radiation reaching the earth (Hollósy, 2002). Ozone is considered the only gas of the atmosphere that absorbs wavelengths shorter than 300 nm (Caldwell *et al.*, 1989). Therefore, most of the published research has focused on effects of elevated UV-B radiation on ecosystems and plants in particular.

Researchers have also investigated the potential benefits of radiation on plant growth induction, plant behavior and acclimation (Darras *et al.*, 2015; Qi *et al.,* 2014; Singh and Datta, 2010). Since the discovery of ultraviolet waves, scientists have attempted to study the impact of these rays on seeds and plant growth (Krizek, 1975; Noble, 2002; Popp and Brown, 1933). UV irradiation of seeds produces free radicals which change cell membrane permeability and electric potential, presumably initiating diverse metabolic responses (Rogozhin *et al.,* 2000). On the other hand, UV irradiation can affect DNA resulting in several photoproducts and pyrimidine dimers (Ravanat *et al.,* 2001; Sancar and Sancar, 1988). It was reported that pre-sowing treatments of seeds using high energy radiation like laser, magnetic field and UV will eventually increase corps productivity by enhancing germination and seedling growth (Iqbal *et al.,* 2016; Qiu *et al.,* 2008; Thomas and Puthur, 2017).

Seeds priming by treating seeds with natural or synthetic compounds before sowing is performed so that seeds reach a physiological state where induction for stress tolerance and enhanced plant growth is achieved (Thomas and Puthur, 2017). Seeds priming enhances quality through the activation of pre-germinative metabolism including antioxidant functions and DNA repair mechanisms (Araujo Sde *et al.,* 2016). Studies showed that plants grown from primed seeds exhibited higher tolerance against biotic andabiotic stresses (Borges *et al.,* 2014; Iqbal *et al.,* 2016; Mariz-Ponte *et al.,* 2018; Ouhibi *et al.,* 2014; Rashid *et al.,* 2015; Zhang *et al.,* 2016b). The aim of this work is to investigate the effect of pre-sowing UV-irradiation on germination and vigour under normal and stress conditions. This work will also study the expression of key growth and stress-related genes during the early stages of germination after UV treatment.

MATERIALS AND METHODS

UV irradiation platform setup

The irradiation of cotton seeds was performed using a mercury arc lamp setup that deliver high dose rate of ultraviolet radiations and visible light. This irradiation setup is mainly composed of the mercury arc lamp as the UV source (USH-102D, USHIO, Japan) with power supply (ORIEL, Model Nr. 69907, Newport, Stratford, CT, U.S.A.), a reflecting metallic mirror and suitable support for the studied sample. Figure (1) shows a schematic diagram of the irradiation experimental setup. The beam is collimated by an adapted optical system of lenses and a reflecting mirror integrated in the arc lamp lantern. An Aluminium mirror was used in order to apply vertical irradiation over studied seed samples. Used optical elements permit to realize a quasi-parallel and homogenous UV and visible beam at the samples position (Beam diameter ≈ 5 cm).The optical distance between irradiated sample and the Hg arc lamp position is about 50 cm. The irradiation level was measured at the position of studied seed samples using specific UVC, UVB and UVA radiometers (Optometer P9710, Gigahertz, Germany) and luxmeter (Testo 545, Testo GmbH & Co., Germany). Table (1), states the applied UVC, UVB and UVA radiation doses per one minute of exposure time and the visible light level at the position of irradiated sample. The stability of the applied beam was verified during all irradiation steps.

Seeds' irradiation conditions

Four irradiation periods were chosen (4, 8, 12 and 16 min) and, at each exposure period, five seeds were

irradiated together. The temperature and relative humidity in proximity to the irradiated seeds were registered. The ambient temperature and relative humidity of irradiated samples were maintained at 22°C ± 2 and 35 % ± 3 in order.

Table 1, Applied UV radiations dose (per 1 min exposure time) and visible light level.

UVC [kJ.m^{-2}]	UVB [kJ.m^{-2}]	UVA [kJ.m^{-2}]	Visible [FC]
0.84 ± 0.04	5.10 ± 0.26	10.50 ± 0.53	5390 ± 250

Plant material and cultivation

Plant material consisted of seeds of two *Gossypium hirsutum* L. accredited local variety Deir Al Zour 22 (a selected line from Delta Pine 41). It is noted, through repeated trials and experience that seeds vigour decrease by time of storage. Germination in the field reached 30 % using two years old seeds. Seeds were provided by the Cotton Research Administration (CRA) and were stored at 4 °C for five years. In pots experiments, irradiated cotton seeds were sown (5 seeds) in pots (7 X 9 cm) which contain universal potting soil (3 pots per treatment). Pots were kept in a growth cabinet under 16 hrs light and a temperature of ~ 25 °C. *In vitro* culture of irradiated cotton seeds was conducted using Murashige and Skoog (MS) media supplemented with MS vitamins (4.4 g/L), agar (9 g/L), and sucrose (30 g/L). Testing the effects of both salt and drought stress on irradiated and control cotton seeds were performed *in vitro* at 25 ºC and 16 hrs light. The salt treatment included 75, 125 and 175 mM NaCl and the drought treatment included the application of PEG 6000 to generate osmotic pressure of -0.2, - 0.5 and -1 bar. Fifteen tubes (one seed per tube) were used for each treatment.

Flow Cytometry

The method described by Jin *et al.* (2008), was used to analyze nuclear DNA content to determine the variations in cell cycle due to UV treatments (Jin *et al.,* 2008). Samples used for flow cytometry were dry and non-irradiated cotton seeds, dry and irradiated (4, 8, 12, and 16 min) cotton seeds, 2 DAP (days after planting) roots from non-irradiated cotton seeds, 2 DAP roots from irradiated (4, 8, 12, and 16 min) cotton seeds, 6 DAP roots from non-irradiated cotton seeds, 6 DAP roots from irradiated (4, 8, 12, and 16 min) cotton seeds.

Plant tissues (seeds, and roots) were rinsed with 2% filtered bleach and washed thoroughly with sterile filtered deionized water. The plant cells nuclei in those tissues were mechanically extracted by chopping with scalpel in the presence of Ice-cold extraction buffer (70 mM NaCl, 0.2 mM EDTA-acetic acid, 0.1M Tris, 0.5% (v/v) Tween20, pH 7.5). For each sample, nuclei preparation was filtered through a 50-µm nylon mesh, followed by centrifugation at 500 xg (for 10 min, at 4°C). Then the supernatant was discarded and cell nuclei were suspended in 1 ml extraction buffer containing propidium iodide (50µg/ml). This preparation was then analyzed on BD Biosciences FACSCalibur™ flow cytometer (BD Biosciences, USA) with doublet discrimination module on. At least 10,000 nuclei were analyzed per sample. Three samples (replicates) for each treatment were analyzed. Flow cytometric data acquisition was performed using CellQuest software (BD Biosciences, USA). Cell cycle data analysis was done using ModFitLT V3.2 software (Verity Software House, USA).

Gene expression analysis

Gene expression analysis of each of *VPP*, *DEH* and *GA3ox1* genes was conducted using cDNA synthesized from RNA of the following samples: UV non-treated cotton seeds in three stages (dry, 1, and 3 DAP), and 12 min UV irradiated seeds in three stages (dry, 1, and 3 DAP). Seeds were planted in wet potting compost in potting trays and were irrigated daily.

Total RNA was isolated following a modified and rapid isolation protocol (Jawdat and Karajoli, 2012) and DNA contamination was eliminated using the TURBO DNA-free™ (Ambion/life technologies, USA) following the manufacturer's manual. The iScript™Select cDNA Synthesis kit (Bio-Rad, USA) was applied to produce, cDNA starting from 1 µg RNA per sample. Real-Time PCR was performed in two replicates in a 25 µl of a

reaction mixture composed of 12.5 µl IQ SYBR super mix (BioRad, USA), 1 µl of cDNA, 1 µl of each of the forward and reverse primer (10 µM), and 9.5 µl of d.d. water. The quantification of mRNA levels was normalized with the level of mRNA for *GhEF1α5* (Artico *et al.,* 2010). The relative expression (fold expression) was calculated using the $\Delta\Delta C_t$ method as follows:

$$2^{-\Delta\Delta Ct} = 2^{-((Ct\ treated_{target\ gene} - Ct\ treated_{reference\ gene}) - (Ct\ untreated_{target\ gene} - Ct\ untreated_{reference\ gene}))}$$

Specific target and reference gene primers are listed in table 2,

Real-Time PCR reaction were performed using the following cycling conditions: initial cycle of 95 ºC for 4 min, followed by a 35 repeated cycle of 95 ºC for 30 sec, 55 ºC for 45 sec, and 72 for 45 sec. The final cycle was at 72 ºC for 7 min.

Statistical analysis

Statistical data analysis was conducted using Analysis of Variance (ANOVA) at 0.05 significance level (STATISTICA 6.1, StatSoft, 2003).

RESULTS

UV irradiation exposure period

Four exposure periods (4, 8, 12 and 16 min) were tested. Germination of long-term stored cotton seeds was observed in pots that were kept in a growth cabinet under 16 hrs light a day and a temperature of ~ 25 °C, interrupted with a 10 °C for about three weeks early on the experiment. The germination of non-irradiated seeds was 13 % in the 3rd week after sowing and kept in the same percentage in the 7th week. However, in the 3rd week after sowing, seed germination was about 7 % in all levels of irradiation. Germination continued and showed varied percentage among the different levels of irradiation in the 7th week. It reached 46 % for each of the 4 and 8 min exposure period; and 40 and 33 % for the 12 and 16 min exposure times. Seedlings were observed until giving the second true leaf and all showed normal development behaviour.

Flow Cytometric cell cycle analysis

Data showed gradual increase in the percentage of cells in the S phase from day zero to the 6th DAP roots of seedlings germinated from seeds subjected to 8 min and 12 min of UV-irradiation. Non-irradiated seeds and 4 min irradiated seeds showed a decrease in root S phase cells on day 6 after planting. Exposure to 16 min of UV irradiation resulted in the least percentage of cells in the S phase in both 2nd and 6th DAP roots. Cell arrest at G2/ M phase was clearly noted in the 6th DAP root samples coming from seeds exposed to 16 min UV-irradiation (Fig. 2). Representative flow cytometric cell cycle analysis is shown in figure 3.

UV-irradiation effects on germination under stress conditions

Two exposure periods, 8 and 12 min were selected to test their effects on seeds germination under NaCl and PEG-induced stress. Seeds were germinated *in vitro* on MS media supplemented with 75, 125 and 175 mM NaCl and on MS media supplemented with PEG 6000 to generate osmotic pressure of -0.2, -0.5 and -1 bar.

Twenty DAPs Plant height showed a significant decrease in plants originated from both irradiated and non-irradiated seeds grown in media supplemented with 125 and 175 mM NaCl compared to plants grown in media with 75 mM NaCl and the control media (salt-free). Seeds exposure for 12 min UV has induced minor increase in plant height compared to the 8 min exposure. Plants coming from seeds irradiated with 8 min UV and grown in control media, showed a significant increase in height compared to non-irradiated plants. Similar pattern has been observed concerning root length and number of true leaves, except that there were no true leaves on plants grown on 125 and 175 mM NaCl supplemented MS media (Fig. 4).

UV irradiation for 8 min in our experiments, has proved repeatedly to enhance germination and growth in control MS media compared to non-irradiated and 12 min irradiated plants. Exposure to 12 min UV showed growth induction under – 0.2 and – 0.5 bar compared to non-irradiated and 8 min irradiated plants (Fig. 5). Germinated plants, with roots and open cotyledon leaves, under – 1 bar have submerged and sank into the media and were excluded from data readings.

Gene expression analysis

Two stress-related genes (*DEH* and *VPP*) and the gene involved in the production of bioactive gibberellins (*GA3ox1*), were selected for gene expression analysis.

Data analysis showed a significant increase in *DEH* expression in mature dry seeds after exposure to UV radiation for 12 min compared to non-irradiated seeds. The *DEH* gene expression decreased gradually from the 1st DAP till the 3rd DAP in both irradiated and non-irradiated seeds (Figure 6-A).The *VPP* gene showed a similar pattern in non-irradiated seeds, showing a significant decrease in expression on 1st and 3rd DAP compared to its expression in dry seeds. A non-significant decrease in expression was noted in dry seeds after irradiation compared to non-irradiated seeds. Notably, gene expression of *VPP* showed a significant increase on the first day of planting compared to non-irradiated seeds planted on the same day. On the 3rdDAP, a decrease in *VPP* expression was noted in irradiated seeds to a level that showed no significant difference from the non-irradiated seeds (Figure 6-B).

The gene *GA3ox1* is involved in the production of bioactive gibberellic acid and hence seed germination and post embryogenic developments of plants (Gupta and Chakrabarty 2013; Koornneef *et al.*, 2002). Gene expression analysis showed a significant increase in both irradiated and non-irradiated seeds on the 3rd DAP compared to dry and 1st DAP seeds (Figure 6-C).

Table 2. Specific target and reference genes primer sequences

Gene	GenBank accession No.	Forward and reverse primers (5′-3′)
pyrophosphate-energized vacuolar membrane proton pump gene (*VPP*)	HM370494.1	F-TGGTTTATTGGTACTTTACATTGCC
		R-CCTTTCAACCTTTCCTACGA
		R-ACAATCATCTTCCTCACTTC
Gossypium hirsutum dehydrin (Lea3-D147) gene (DEH)	M81655.1	F-ATGGCACACTTTCAGAATCA
		R-TAGAGGAACTTCCAGTACGA
Gossypium hirsutum gibberellin 3-beta-dioxygenase 1 (GA3ox1)	HQ891936.1	F-ATTATTAGACTTCATCCTGATTTCACTTCCC
		R-GATTCAACCATGTCAAGTAGAGTAGTGGAG
*Elongation Factor 1-alpha*gene (*Ef1α5*)	DQ174254	F-TCCCCATCTCTGGTTTTGAG
		R-CTTGGGCTCATTGATCTGGGT

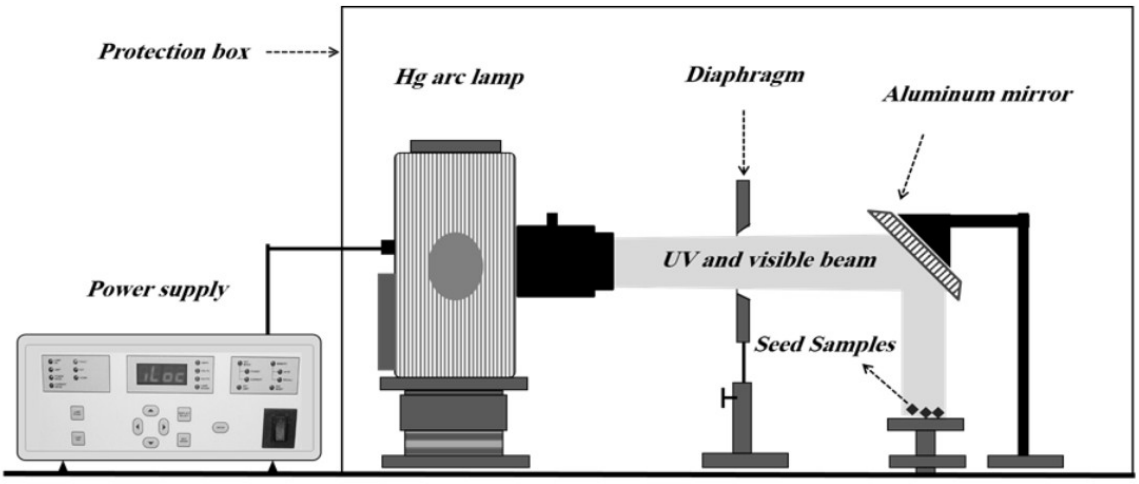

Figure 1. Illustration of the UV irradiation experimental setup

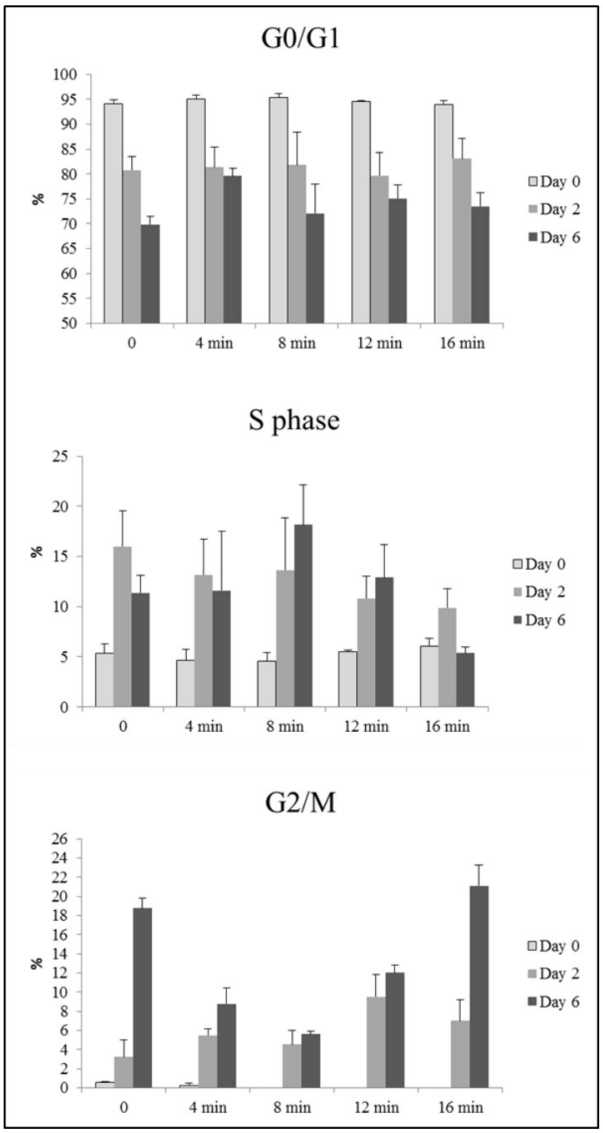

Figure 2. Flow cytometric cell cycle analysis data of dry mature seeds and 2nd and 6th DAP roots samples coming from non-irradiated seeds and seeds subjected to 4, 8, 12, 16 minutes of combined UV-A, B and C radiation

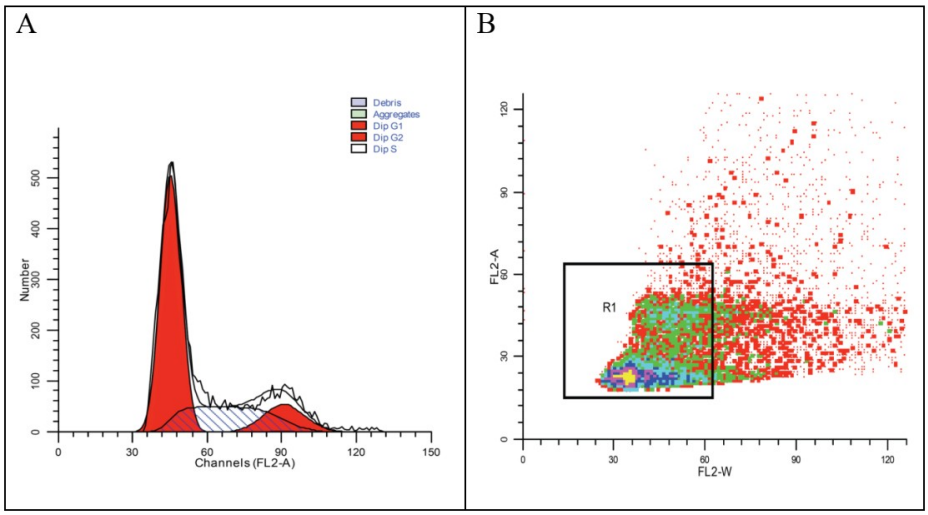

Figure 3. Representative data of cell cycle analysis of *Gossypium hirsitum* var. Deir Al-Zour 22. The DNA histogram (A) of gated events (nuclei) in (B)

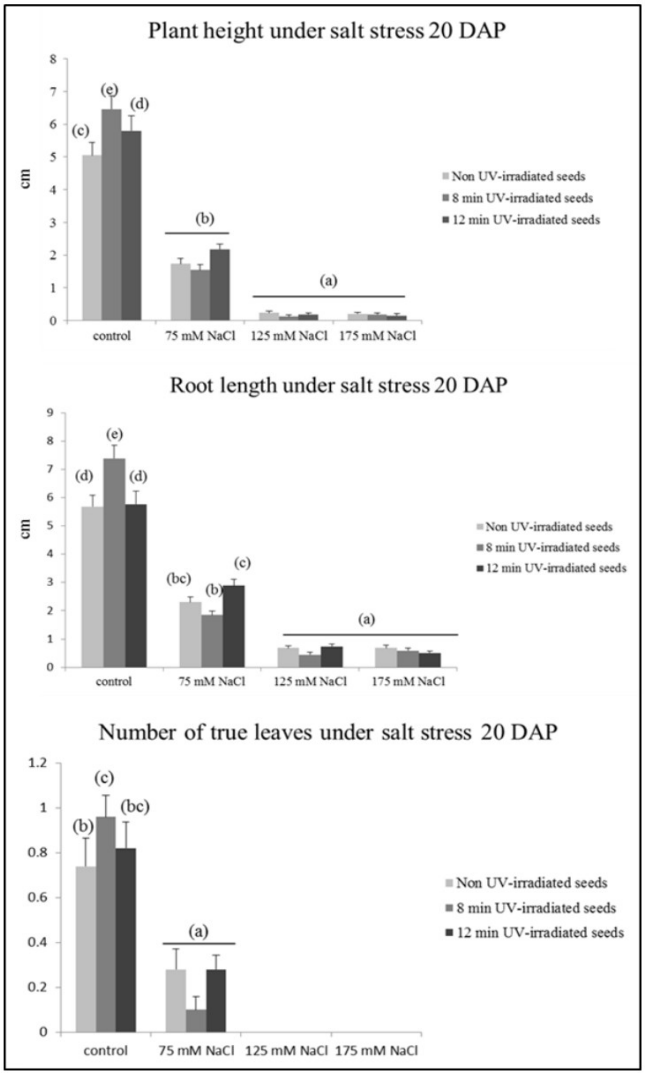

Figure 4. Twenty days seedlings coming from UV-irradiated (8 and 12 min) and non-irradiated seeds. Seedlings were grown on MS media supplemented with 0, 75, 125 and 175 mM NaCl. Data include plant height, root length and number of true leaves. Data represent means and standard error of 10 replications. Data were subjected to Duncan's test with a confidence level of 95% using STATISTICA program. Bars sharing a letter are not significantly different

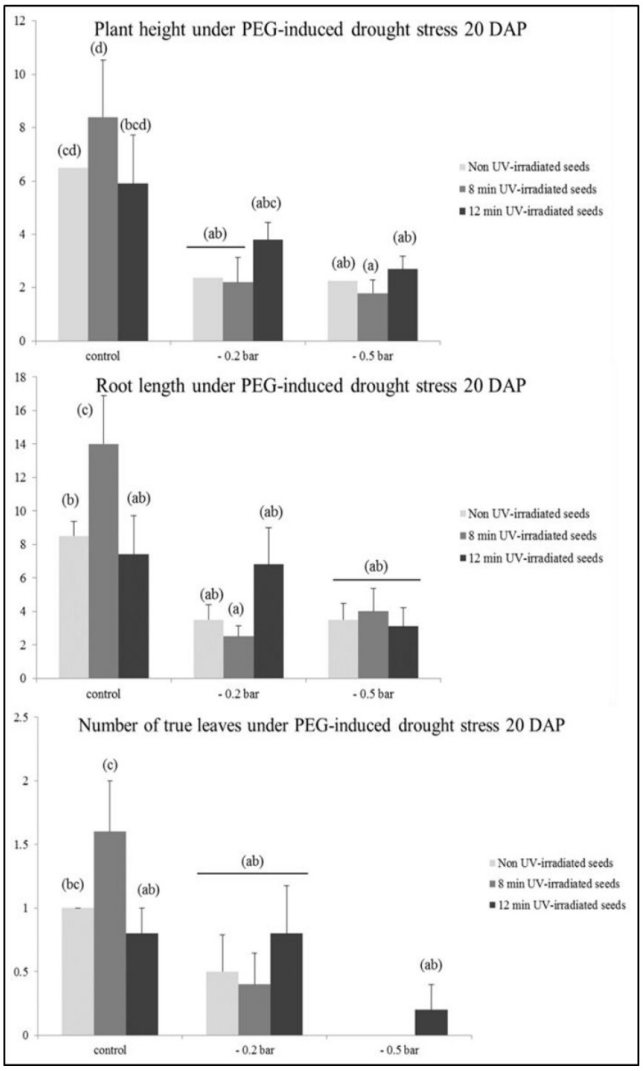

Figure 5. Twenty days seedlings coming from UV-irradiated (8 and 12 min) and non-irradiated seeds. Seedlings were grown on control MS media and PEG-6000 supplemented media to create – 0.2 and – 0.5 bar. Data include plant height, root length and number of true leaves. Data represent means and standard error of 10 replications. Data were subjected to Duncan's test with a confidence level of 95% using STATISTICA program. Bars sharing a letter are not significantly different

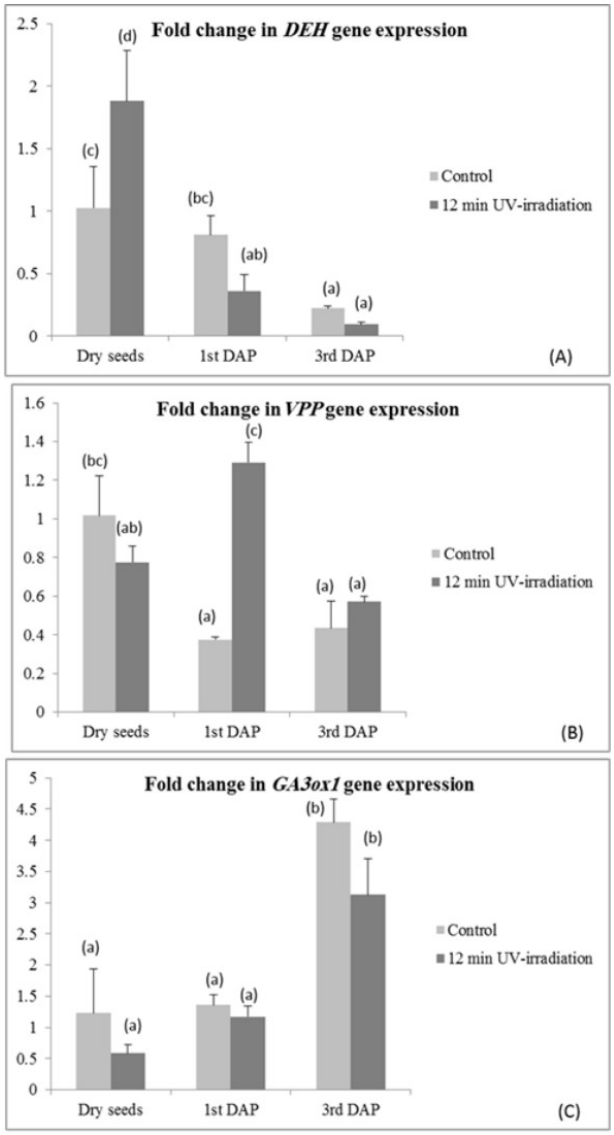

Figure 6. Fold expression of *DEH*, *VPP* and *GA3ox1* genes in dry mature seeds and in 1st and 3rd DAP germinated seeds. Samples were prepared from non-irradiated and UV-irradiated seeds. Data were subjected to Duncan's test with a confidence level of 95% using STATISTICA program. Significant fold expression change of *DEH* and *VPP* genes was observed between irradiated and non-irradiated seeds in dry and 1st DAP seeds, respectively. Data represent means and standard deviation of three replications

DISCUSSION

The increased amounts of UV radiation in the atmosphere are expected to have negative effects on plant behaviour (Zhu and Yang, 2015). However, the priming effects of low levels of UV radiation on plant growth, defence and tolerance have been reported in several plant species. Enhancement of seed germination, chlorophyll synthesis, and nitrogen and protein content are some of these effects which would overall contribute to stress tolerance (Ouhibi *et al.*, 2014; Rai *et al*., 2011; Thomas and Puthur, 2017).

In the present study, dry mature long-term stored cotton seeds were exposed to considerably low doses of combined UV-A, B and C radiation. The results showed enhanced germination rates under applied UV exposure periods (4, 8, 12, and 16 min) and specifically under 8 min compared to non-irradiated seeds. Germination induction can be due to the higher energy of UV radiation, and hence a larger effect on seed coat allowing germination (Kovacs and Keresztes, 2002;

Noble, 2002). However, UV-B radiation can lead to growth inhibition by inducing the formation of pyrimidine photodimers such as, cyclobutane pyrimidine dimers (CPDs) which induce cell arrest. Photodimers are repaired by photolysis, specific to each photodimer, which are activated by UV-A/blue light (Takahashi *et al.*, 2015). In our study, the effects of the UV irradiation on seeds germination and cell cycle can be due to the combined UV-A, B and C in which a possible synchronized balance is taking place between the production of photodimers under UV-B and photolysis activated by the UV-A. Plants from germinating seeds treated with 8 and 12 min UV, and specifically 8 min, were induced and showed a continuous increase in percentage of cells in the S phase in preparation for cell division. On the other hand, the samples subjected to 16 min UV showed a normal 2nd DAP behaviour, but cell percentage at the S phase declined on the 6th DAP. This might indicate that the repair mechanisms in germinating seeds subjected to higher doses of UV irradiation are not yet complete or that replication is destabilized, which will eventually prevent cells from dividing (Francis, 2011).

Our results have also shown that the exposure of seeds to 12 min UV showed better plant growth and biomass than each of the control and 8 min UV-irradiated samples, under moderate PEG and NaCl induced stresses. Several reports discussed the impact of UV radiation on plant growth and biomass is diverse and not yet conclusive, a stimulatory in some species and inhibitory in others (Biever and Gardner, 2016; Verdaguer *et al.,* 2017). Most of the published papers have discussed impacts of UV-A, B and C separately (Formica-Oliveira *et al.*, 2016; Gwynn-Jones *et al.*, 2012; Rai *et al.*, 2011; Verdaguer *et al.*, 2017). A study published in 2002 showed that UV-irradiation induced rapid germination, however, the continued UV-irradiation showed damaging effects on germinated plants (Noble, 2002). Mariz-Ponte with coauthors in their research (Mariz-Ponte *et al.*, 2018), investigated the beneficial effects of moderate UV-A supplementation on tomato seeds performance during germination and first stages of seedlings growth. Their data pointed to the 'eustress' beneficial of UV-A supplementation in growth and vigour. Ma with coauthors, were able to reach the balance between the "eustress" and 'distress' caused by UV-B irradiation for maximum isoflavones accumulation in germinated soybeans (Ma *et al.*, 2018). It was proposed by Hideg with coauthors that low UV-B doses causes 'eustress' (good stress) in plants and creating a low alert status which includes the activation of antioxidant responses (Hideg *et al.*, 2012). Seeds with higher content of antioxidants can hold biochemical traits that assist their seedlings to survive under unfavourable conditions (Sahitya *et al.*, 2018). In our work, a Hg arc lamp set-up platform permitted a parallel and homogenous UV beam on seeds samples. Therefore, seeds germination and subsequent seedlings growth in control and stressed conditions, can be a result of collective effects of different UVRs. It can be suggested that the cotton UV-irradiated seeds in our study have experienced an activation of antioxidant responses which may explain the improved growth exhibited by seedlings originating from seeds irradiated with 12 min UV compared to non-irradiated seeds under stress conditions.

Considering the growth improvement effect of the 12 min UV irradiation under stress conditions, gene expression analysis was conducted on plant samples from irradiated and non-irradiated seeds during the first three days of germination. The expression analysis of *DEH*, a stress-related gene in cotton that belongs to the LEA proteins, showed a significant jump when irradiated. This can be due to the DEHs properties of scavenging reactive oxygen species (ROS), a typical by-product of the photo-excitation under UV irradiation, indicating their function as antioxidant molecules (Hanin *et al.*, 2011; Yokawa *et al.*, 2016). The significant increase in expression after irradiation was followed by a gradual decrease in expression in both irradiated and non-irradiated samples after imbibition, which comes in agreement with the fact that these proteins accumulate during late embryogenesis stages and under stress conditions that cause cell dehydration (Hanin *et al.*, 2011; Lee *et al.*, 2005).

Gene expression analysis of the proton transport protein, the vacuolar H^+-PPase, under UV irradiation was also covered in our work. It has been reported that

the level of H^+-PPase varies according to the physiological status and in response to environmental stresses (Maeshima, 2000). The H^+-PPase have a key role in increasing salt and drought tolerance. The Arabidopsis *AVP1*-expressed cotton displayed significant improvement of tolerance to drought and salinity stress (Pasapula *et al.*, 2011). Zhang *et al.* reported that the expression of *TsVP* from *Thellungiella halophila* in transgenic cotton exhibited an improvement of seed cotton yields in saline fields (Zhang et *al.*, 2016a). Up to our knowledge, there are no reports discussing the effects of non-ionizing radiation on the expression of H^+-PPases. Our study showed a significant decrease in expression of *VPP* gene after imbibition in non-irradiated seeds, while the irradiated seeds showed a significant increase. This can be explained by the increased activity of VPP in accumulating ROS, produced under UV irradiation, into vacuoles and hence improved osmoregulation.

The transition from a dormant seed to a seedling through germination is a crucial developmental step in a plant life cycle. It is the state of a balance between abscisic acid, which induces dormancy and gibberellins that promote germination (Boccaccini *et al.*, 2016; Seo *et al.*, 2008). The GA biosynthetic gene *GA3ox1* and *GA20ox* are the main catalysts in the late steps of GA biosynthetic pathway (Seo *et al*., 2008). Light regulates biologically active gibberellins levels through regulating the function of several genes including *GA3ox1* (Oh *et al.,* 2006; Toyomasu *et al.,* 1998). Studies reported the inhibiting effect of far red light on expressed gibberellins produced under red-light (Oh *et al.*, 2006; Seo *et al.*, 2008). To our knowledge, no studies investigated the effect of direct UV irradiation on the expression of *GA3ox1* gene. Our study investigated the effect of UV irradiation for 12 min on the expression of *GA3ox1* and results showed a significant increase in expression on 3rd DAP, which supports the *GA3ox1* role in germination. However, UV irradiation showed a neutral effect on seeds behaviour compared to non-irradiated seeds. This may suggest that UV irradiation showed no induction or inhibition in the early stages of germination, and will need further investigation on UV longer exposure effects on germination induction.

CONCLUSION

The application of combined UV A, B, and C irradiation on long-term stored seeds of cotton showed to be inductive using 8 min exposure in normal growth conditions and 12 min in PEG and NaCl supplemented media. An increase in cells percentage in preparation for division was clearly noted under 8 min UV-irradiation. However, the higher the UV-irradiation exposure, the larger the percentage of cell arrest. The UV-irradiation showed no harmful effects on the expression of *GA3ox1* gene, suggesting normal levels of gibberellins controlling the transition from seed dormancy to germination. Two stress-related genes *DEH* and *VPP* were temporarily activated after treatment with UV-irradiation supporting their function as scavenging and accumulating factors of ROS, a typical by-product of the photo-excitation under UV. Our results support our hypothesis that combined UV-A, B and C can be applied as a physical priming agent to induce plant vigour and enhance germination in stressed condition without affecting normal growth.

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