



Research article

Differential responses of pea seedlings to salicylic acid under UV-B stress

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Abstract: In nature, plants are continuously exposed to solar light. They cannot avoid exposure to UV-B radiation. The purpose of this investigation was to examine how UV-B radiation affects seed germination, seedling growth, protein and sugar contents and activities of antioxidant enzymes in pea (*Pisum sativum*) seedlings. Salicylic acid mediated physiological responses in UV-B stressed pea seedlings. UV-B exposure adversely affected germination and physiology of *Pisum sativum* L. Salicylic acid mitigated the impacts of UV-B stress. Seed germination decreased with increased duration of UV-B exposure. Enhanced activities of antioxidant enzymes in response to UV-B radiation played a protective role against UV-B radiation.

Keywords: Antioxidants - Oxidative stress - *Pisum sativum* L. - UV-B radiation - Salicylic acid.

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INTRODUCTION

Plants are exposed to various abiotic and biotic stresses including solar ultraviolet-B (UV-B) radiation in the natural environment. UV-B radiation is the biggest challenge to life forms on the earth. Over the last few decades degradation of ozone layer leads to enhanced solar UV-B radiation on the earth (McKenzie *et al.* 2003, Liu *et al.* 2013). The damage of the ozone layer caused by human activities may result in increased level of ultraviolet radiation reaching the earth surface which is harmful for all living beings including crop plants (Shaukat *et al.* 2011). UV-B exposure alters biochemical processes in crop plants and affects the morphological parameters (Casati & Walbot 2003, Zu *et al.* 2010). The exposure of plant to UV-B damages macromolecules viz., nucleic acid, lipid and proteins (Ries *et al.* 2000, Frohnmeyer & Staiger 2003) decreases photosynthetic rate (Feng *et al.* 2003) and alters activities of several antioxidant enzymes (Agrawal & Mishra 2009). The UV-B radiation has adverse impact on growth and decreases productivity of crop plants (Shaukat *et al.* 2011).

Plant growth regulators mitigate adverse effects of various environmental stresses. Salicylic acid (SA), a secondary phenolic metabolite, is considered as plant hormone. It is naturally found in plants and acts as a signaling molecule (Davies 2004, Amin *et al.* 2013). SA plays an important role in regulating the metabolic activities of plants (Davies 2004, Amin *et al.* 2013). SA application enhances the biomass production and yield in a variety of plants like maize (Amin *et al.* 2013), wheat (Arfan *et al.* 2007) under adverse conditions. SA also activates the defense system of plants to protect them from deleterious impact of abiotic stress.

Pisum sativum L. (pea) is one of the most economically important pulse crops in India because of its high protein content. Cultivation of pea is common in India due to its ability to fix atmospheric nitrogen. It is used as soil primer for other crops.

The aim of the present study was to examine the impact of UV-B exposure on seed germination and growth of pea and the stress responses of SA in UV-B stressed pea seedlings. This study may help regulate the tolerance of abiotic stress like UV-B by plant growth hormone.

MATERIALS AND METHODS

Seeds and chemical

Seeds of *Pisum sativum* L. cv. Rachana were procured from the Seed Agency at Allahabad, Uttar Pradesh,

India. Salicylic acid (molecular weight 138.121 g.mol⁻¹) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India.

Salicylic acid and ultraviolet-B treatments

Salicylic acid was prepared by dissolving a requisite amount of SA in 1.0 mL of ethanol and the volume was made 100 mL with double distilled water and used for treatment. Seeds were exposed to UV-B radiation for 15, 30, 60 and 90 min. Fluorescent UV-B tube (TL 40 W/12 Philips, Holland) was used for UV-B irradiation. The UV-B tube was wrapped with cellulose acetate filter (Johnston Industrial Plastics, Toronto, Canada) to avoid all incidents of UV-C (< 280 nm). The UV-B irradiation intensity was measured with the help of power meter (Spectra Physics, USA model 407, A-2).

Petri-plate assay

Seeds were soaked in two groups each in 100 mL of SA (0.5 mM). The seeds soaked in double distilled water (DDW) for 4 hours, separately. After completion of course of time the seeds of the two groups were further divided into five sets. Out of five, four sets of each group was exposed to UV-B radiation for 15, 30, 60 and 90 min. The complete experimental setup has ten different combinations such as: control (without treatment), SA (0.5 mM), UV-B₁ (15 min), UV-B₂ (30 min), UV-B₃ (60 min), UV-B₄ (90 min), UV-B₁+SA, UV-B₂+SA, UV-B₃+SA, UV-B₄+SA. Ten seeds of each treatment were placed at equal distance in sterilized petriplates (dia 9 cm, depth 1.5 cm) lined with double layer of Whatman No. 1 filter papers. Filter papers were moistened with 5 mL of SA for respective treatments and DDW in control and incubated at 28°±2° C for germination in growth chamber. The experiment was conducted in replicate of three. Germination was initiated at 48 hrs after sowing. Germination and seedling growth were recorded till 7 days after sowing at the interval of 24 hours. Radicle and plumule length was recorded with the help of metric scale.

Protein content

Protein content was determined according to the method of Lowry *et al.* (1951). The amount of protein was calculated with reference to the standard curve obtained from bovine serum albumin.

Sugar content

The determination of total soluble sugars (TSS) was done following Hedge & Hofreiter (1962). About 0.1 g radicle was homogenized in 5 mL 95% (v/v) ethanol. After centrifugation, 1 mL supernatant was mixed with 4 mL anthrone reagent and heated on boiling water bath for 10 min. Absorbance was recorded at 620 nm after cooling. The amount of sugar was determined by the standard curve prepared from glucose.

Extraction and assay of antioxidant enzymes

Fresh sample of radicle (0.25 g) was homogenized with 0.1 M sodium phosphate buffer containing 1% (w/v) polyvinyl pyrrolidone (pH 7.0) in a pre-cooled mortar and pestle. The extract was centrifuged at 4° C at 14,000g for 30 min in cooling centrifuge (Remi instruments C 24). The supernatant was used for the assay of antioxidant enzymes *viz.*, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities.

The SOD activity (EC 1.15.1.1) was estimated by the nitroblue tetrazolium (NBT) photochemical assay following Beyer and Fridovich (1987). The reaction mixture (4 mL) consisted of 20 mM methionine, 0.15 mM ethylene diamine-tetra acetic acid (EDTA), 0.12 mM NBT and 0.5 mL supernatant. The test tubes were exposed to fluorescent lamps for 30 min and identical unilluminated assay mixture served as blank. One unit of enzyme was measured as the amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase (CAT, EC1.11.1.6) activity was assayed following the method by Cakmak & Marschner (1992). Assay mixture (2 mL) contained 25 mM sodium phosphate buffer (pH 7.0), 10 mM H₂O₂, and 0.5 mL enzyme extract. The rate of H₂O₂ decomposition for 1 min was monitored at 240 nm and calculated using extinction coefficient of 39.4 mM⁻¹cm⁻¹ and expressed as enzyme unit mg⁻¹ protein. One unit of CAT was determined as the amount of enzyme required to oxidize 1 mM H₂O₂ min⁻¹.

Ascorbate peroxidase (APX, EC1.11.1.11) activities were assayed following Nakano & Asada (1981). Assay mixture (2 mL) contained 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H₂O₂, and 0.2 mL enzyme extract. H₂O₂ was the last component to be added. The absorbance was recorded for 1 min at 290 nm (extinction coefficient of 2.8 mM⁻¹cm⁻¹). Enzyme specific activity was measured as enzyme unit per one milligram protein as the amount of enzyme required to oxidize 1 mM H₂O₂ min⁻¹.

Guaiacol peroxidase (GPX, EC 1.11.1.7) activities were assayed following Hemeda and Klein (1990). The reaction mixture (2 mL) contained 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.05% (v/v) guaiacol, 1.0 mM H₂O₂, and 0.2 mL of enzyme extract. The increase in absorbance due to oxidation of guaiacol was monitored at 470 nm. The enzyme activities were measured using extinction coefficient of 26.6 mM⁻¹cm⁻¹ and expressed as enzyme unit per mg protein.

RESULTS

UV-B decreased seed germination in dose dependent manner. However, in combination with SA gradual increase in germination was recorded (Table 1). Maximum 53.84% reduction in seed germination was observed in the pea seedlings exposed in UV-B for 90 min. Growth was measured in terms of radicle and plumule length. UV-B radiation induced impact on growth of pea seedlings was measured and results are presented in tables 1. UV-B significantly declined the radicle and plumule length which was concentration dependent. Maximum 59.11% and 90.66% decrease in radicle and plumule length in the pea seedlings over control was recorded respectively in 90 min single UV-B treatment. Application of SA with UV-B exposure exhibited positive effects on radicle and plumule length of the seedlings.

Table 1. Mitigating effects of salicylic acid on UV-B stressed *Pisum sativum* L.

Treatments	Seed germination %	Radicle length (cm)	Plumule length (cm)	Protein (mg.g ⁻¹ FW)	Sugar (mg.g ⁻¹ FW)
C	97.5±0.21a	9.05±0.31b	3.75±0.02b	21.47±0.05b	29.66±0.26b
S	100.0±0.23a	10.3±0.57a	4.4±1.0a	25.80±1.48a	32.80±0.29a
UV ₁	82.5±0.33b	7.85±0.20c	2.05±0.60bc	16.34±0.36c	28.20±0.53bcd
UV ₂	72.5±1.43d	7.1±0.17c	1.65±0.83c	9.33±1.48d	26.33±0.26de
UV ₃	57.5±1.33f	5.2±0.05de	1.25±0.54de	6.03±0.014e	24.10±0.27fg
UV ₄	45.0±0.76g	3.7±0.34f	0.35±0.14e	4.23±0.014f	20.67±0.26h
UV ₁ +S	85.0±0.32b	8.1±0.41c	3.4±0.28abc	19.34±1.45b	29.16±0.02bc
UV ₂ +S	77.5±1.43c	7.85±0.02c	2.6±0.80cd	10.66±1.47d	27.64±0.08cde
UV ₃ +S	62.50±1.3e	6.1±0.23d	2.7±0.11cd	7.95±0.34e	25.72±0.20ef
UV ₄ +S	55.0±0.67f	4.35±0.54ef	0.55±0.25e	5.75±0.08f	22.59±0.32g

Note: Mean±SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test), n = 3. C= control, S= 0.5mM concentration of salicylic acid, UV₁= 15, UV₂ = 30, UV₃ = 60 and UV₄ = 90 min treatment of ultraviolet-B radiation, UV₁+S, UV₂+S, UV₃+S and UV₄+S are combined treatments of UV-B and salicylic acid.

The results pertaining to protein and sugar in seedlings are depicted in table 1. The increase in duration of UV-B radiation from 15 min to 90 min caused progressive decrease in protein and sugar contents. A steep decline of 80.29% protein and 30.33% sugar in pea was recorded following 90 min of UV-B exposure.

The seedlings showed significant increase in activities of antioxidant enzymes viz., SOD, CAT, APX and GPX following UV-B exposure from 15 to 90 min (Fig. 1). The seedlings exposed to UV-B alone exhibited an increase in the activities of SOD, CAT APX and GPX by 59.78%, 88.76%, 81.56% and 62.80% respectively over the control. Simultaneous treatment with SA and UV-B caused significant improvement in the activities of SOD, CAT, APX and GPX. SA manifested positive impact on UV-B stressed seedlings to avoid oxidative damage caused by UV-B exposure.

DISCUSSION

UV-B radiation is one of the important abiotic stress factors manifesting significant impact on growth and physiological processes in plants (Mishra *et al.* 2009, Dwivedi *et al.* 2015). This problem is aggravated due to further increase in UV-B radiation in troposphere. Previously it has been demonstrated that SA priming reduced susceptibility towards different kinds of abiotic stresses by modulating defense system (Horváth *et al.* 2007) Decline in seed germination under UV-B exposure was reported (Shaukat *et al.* 2011). Our results are in agreement with the previous findings of Shaukat *et al.* (2011) on *Helianthus annuus*. SA in low doses significantly increased seed germination in *Arabidopsis* under different abiotic stresses (Rajjou *et al.* 2006). The positive effect of SA on seed germination under abiotic stress is due to decreased level of oxidative damage (Peykarestan *et al.* 2012) and it also initiates translation and elongation factors, proteases and two subunits of the 20S proteasome which consequently revamp seed germination by encouraging protein synthesis that are indispensable for seed germination and the movement or protein breakdown gather during the course of seed

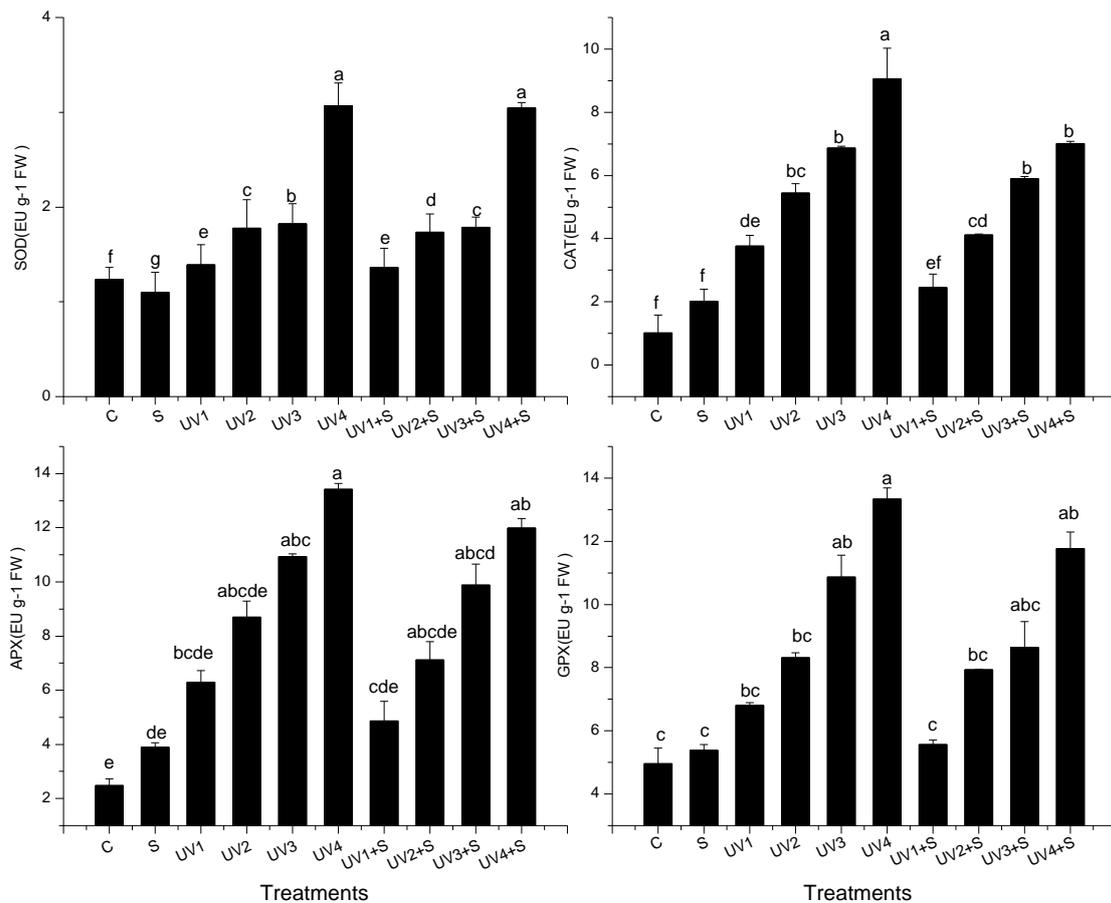


Figure 1. Mitigating effects of salicylic acid on antioxidant enzyme activities of UV-B stressed *Pisum sativum* L. Mean±SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test), n =3. C= control, S= 0.5mM concentration of salicylic acid, UV₁= 15, UV₂= 30, UV₃= 60 and UV₄= 90 min treatment of ultraviolet-B radiation, UV₁+S, UV₂+S, UV₃+S and UV₄+S are combined treatments of UV-B and salicylic acid.

maturation and also in the synthesis of various enzymes used in several metabolic pathways like glycolysis, glyoxylate cycle, pentose phosphate pathway, and gluconeogenesis (Rajjou *et al.* 2006). The results of the present study showed that UV-B alone decreased growth of pea seedlings in dose-dependent manner. The declined growth in *Helianthus annuus* exposed to UV-B is reported (Shaukat *et al.* 2011). In contrast, SA priming of seedlings /plants ameliorated the toxic effect of UV-B and promoted growth as compared to the pea seedlings exposed to UV-B treatments alone. SA pretreatment may mask impact of the UV-B response of the seedlings by modulating the antioxidant system. UV-B induced reduction in protein content of pea at higher duration of UV-B exposure may be linked with UV-B induced disturbance in protein synthesis. Like protein content we recorded reduced sugar content under UV-B stress. Seed germination and early development of seedlings exploit their storage food material (Rosa *et al.* 2009) and when plants are exposed to stress, some protective mechanisms can also form in plants and it requires energy and resources and the resource of energy in plants is mostly sugar (Ho 1988). Similar to our results decreased sugar content observed in maize (Correia *et al.* 2005) Different metabolic pathways in plants continuously produced reactive oxygen species as byproduct. Plants exposed to increased UV-B radiation induced ROS accumulation which includes not only free radicals such as superoxides but also hydrogen peroxide and singlet oxygen which caused oxidative damage to nucleic acid and proteins (Foyer *et al.* 1994) and metabolic system. The plants have efficient antioxidative defense system to mitigate the harmful impact of oxidative stress induced by UV-B stress. The plant defense system provides protection against free radicals and reactive oxygen species. SA pre-treatment alleviates the adverse effect of UV-B by modulating activity of antioxidant (Horva'th *et al.* 2007). The activities of antioxidant enzymes induced by UV-B exposure varied with plant species. Generally, activities of antioxidants like SOD, CAT, APX and GPX were found to be increased by UV-B radiation (Mishra *et al.* 2009, Dwivedi *et al.* 2015). SA treatment inhibits oxidative damage by modulating antioxidant enzymes activity and detoxifies superoxide

radicals (Farooq *et al.* 2008). SOD present in different part of cell acts as first line of defense in plant. It acts first on free radicals and converts to hydrogen peroxide, CAT in peroxisomes and APX in the cell as whole have potential to convert hydrogen peroxide into water and oxygen (Noctor & Foyer 1998). Kondo and Kawashima have also demonstrated the enhanced antioxidant activity to UV-B exposure (Kondo & Kawashima 2000). The growth of plant is the result of equilibrium between ROS produced and antioxidant enzymes induced. Enhanced activities of antioxidant evinced the plant tolerance against UV-B radiation. Several studies reported the effect of UV-B and salicylic acid on plants but here we studied the effect of UV-B and salicylic acid on seed germination and metabolic activity of pea seedlings.

CONCLUSION

The present study showed that UV-B exposure exhibited adverse effects on seed germination, seedlings growth of *Pisum sativum* and their defence system. The seed priming with salicylic acid, as plant growth regulator, in appropriate concentration alleviate the adverse effects of UV-B radiation in pea seedlings.

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