

# EFFECTS OF SUPPLEMENTAL ULTRAVIOLET-B RADIATION ON GROWTH AND PHYSIOLOGY OF ACORUS CALAMUS L. (SWEET FLAG)

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Increased UV-B radiation due to depletion of stratospheric ozone has potentially harmful effects on plant growth and development. The present study uses a field experiment to examine the effect of long-term supplemental UV-B radiation at two intensities (+1.8 and +3.6 kJ m<sup>-2</sup> d<sup>-1</sup> above ambient) on the growth and physiology of the medicinal plant *Acorus calamus* L. (sweet flag). Plant height and leaf area were inhibited in a dose-dependent manner, with greater inhibition at the higher dose. At the lower dose the net photosynthetic rate increased, with an increase in stomatal conductance and water use efficiency. Stimulation of physiological functions in plants under the lower dose resulted in increased biomass production. At the higher dose, total chlorophyll content showed no marked variation, whereas carotenoids and UV-B-screening pigment flavonoids increased significantly after treatment. Increased flavonoid content under lower exposure correlates well with higher activity of phenylalanine ammonia lyase, a key enzyme of flavonoid biosynthesis. This study clearly showed that the lower dose of supplemental UV-B promoted rhizome growth in *A. calamus*, perhaps due to improved photosynthesis. Plant defense was stronger under the lower dose.

**Key words:** Ultraviolet-B, *Acorus calamus* L., biomass, growth, pigments, phenylalanine ammonia lyase.

## **INTRODUCTION**

Depletion of stratospheric ozone, caused by pollutants such as chlorofluorocarbons, leads to an increase in the amount of UV-B (280-320 nm) reaching the Earth's surface (Madronich et al., 1998). This increased UV-B radiation will influence the growth and metabolism of terrestrial plants due to their requirement of sunlight for photosynthesis, and even a slight increase in UV-B intensity will have a disproportionately large photobiological effect because it is absorbed by important macromolecules (Jansen et al., 1998). Excess UV-B radiation acts as an environmental stress on plants, altering their physiological functions and ultimately slowing plant growth, damaging photosynthetic pigment and lowering CO<sub>2</sub> assimilation; these alterations reduce biomass productivity (Tevini and Teramura, 1989).

Plant species vary greatly in their response to UV-B. Some plants tolerate enhanced UV-B irradiation by acquiring protective modifications such as increased leaf thickness, higher production of UV-B filters (flavonoids), and stimulated antioxidant activ-

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ity acting to quench free radicals (Caldwell et al., 2003; Agrawal and Mishra, 2008).

The effect of enhanced UV-B on plant growth and physiology has been the subject of a number of studies (Jansen, 2002). Plant morphology is considered a very sensitive indicator of UV-B damage, as compared to biomass reduction, under realistic field conditions of high photosynthetic photon flux density (PPFD) (Barnes et al., 1990). Leaf area and plant height decreased when plants were exposed to enhanced UV-B radiation (Zhao et al., 2003). Morphological characteristics can affect the sensitivity of a species to UV-B radiation by influencing the amount of radiation intercepted (Bornman and Teramura, 1993). Measurements of various other important parameters such as chlorophyll and carotenoid content and levels of UV-B absorbing compounds have also proved to be useful indicators

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**Abbreviations:** UV-BBE – biologically effective UV-B; sUV-B – supplemental UV-B; sUV<sub>1</sub> – lower-dose supplemental UV-B (+1.8 kJ m<sup>-2</sup> d<sup>-1</sup> above ambient); sUV<sub>2</sub> – higher-dose supplemental UV-B (+3.6 kJ m<sup>-2</sup> d<sup>-1</sup> above ambient); DAT – days after transplantation; PAR – photosynthetic active radiation; PAL – phenylalanine ammonia lyase; Pn – net photosynthesis; Cs – stomatal conductance; WUE – water use efficiency.

of UV-B tolerance or sensitivity, since these parameters display the most rapid response to UV-B stress (Greenberg et al., 1997; Agrawal and Rathore, 2007). UV-B radiation also affects plant development, in particular biomass partitioning, carbon assimilation pattern and relative growth traits (Gao et al., 2003; Kakani et al., 2003b).

Experiments on terrestrial plant responses to UV-B mostly concern agricultural crops of high economic importance; studies on medicinal plants in realistic environmental conditions are scarce. Very few studies on the impact of UV-B on aromatic and medicinal plants are available (Karousou et al., 1998; Ioannidis et al., 2002; Nitz and Schnitzler, 2004). UV-B research on medicinal plants has dealt mainly with analyses of the content and composition of their essential oil products, with scant attention to its impact on morphological and physiological parameters (Grammatikopoulos et al., 1998; Maffei and Scnnerini, 2000).

The aim of the present investigation was to study the impact of different doses of sUV-B on some growth and physiological parameters of *Acorus calamus*.

## MATERIAL AND METHODS

#### PLANT MATERIAL

Acorus calamus L. (Acoraceae) is an herb grown in the temperate zone and subtropics, commonly cultivated in India for medicinal use. It is a rhizomatous, perennial, aromatic plant having a sweet fragrance. In the Ayurvedic system of medicine, the rhizomes and its essential oil are considered to have strong antispasmodic, carminative and anthelmintic properties, commonly used in epilepsy and mental diseases.

#### STUDY AREA AND PLANT MATERIAL

The field experiments were conducted in the Botanical Garden, Department of Botany, Banaras Hindu University (29°18'N, 81°19'E, 79 m a.s.l.), Varanasi, India. Rhizomes of *A. calamus* collected from the nursery of the B.H.U. Horticulture Department were transplanted to the experimental field in November 2006. Equal-sized rhizomes were transplanted 20 cm apart in  $1 \times 1$  m<sup>2</sup> plots. Mean temperatures during the experimental period were 14.3–17.6°C minimum and 29.8–32.5°C maximum, and relative humidity ranged from 53% to 85%. Photosynthetic active radiation (PAR) during the experimental period ranged between 1100 and 1200 µmol m<sup>-2</sup> s<sup>-1</sup>. Field plots were watered regularly and uniformly to maintain an optimum water regime.

## EXPERIMENTAL DESIGN AND UV-B TREATMENT

Plants were exposed to supplemental UV-B (sUV-B) irradiation with 40W fluorescent UV-B-313 lamps (Q-Panel, Cleveland, OH, U.S.A.). sUV-B treatment was started immediately after the plants established at 20 days and continued up to 100 days for 3 h daily, centered around solar noon (10.00 a.m to 1.00 p.m.).

The experiment consisted of three treatments. Control (C) plants received only ambient UV-B (9.6 kJ m<sup>-2</sup> d<sup>-1</sup> UV-B<sub>BE</sub>). In the sUV-B treatments, UV-B lamps were used to expose the plants to two intensities of supplemental biologically effective UV-B  $(UV-B_{BE})$ : +1.8 kJ m<sup>-2</sup> d<sup>-1</sup> (designated sUV<sub>1</sub> here) and +3.6 kJ m<sup>-2</sup> d<sup>-1</sup> (sUV<sub>2</sub>) above the ambient effective UV-B dose, simulating 5% and 10% reductions of stratospheric ozone at Varanasi (India) at the summer solstice under clear sky. Each treatment was done in triplicate plots. In the control, polyester film (0.13 mm thick) was used to exclude UV-B (eliminates all radiation below 320 nm). In the sUV-B treatment, radiation transmitted by the UV tubes was filtered using 0.13 mm thick cellulose acetate foil (transmission down to 290 nm) to transmit UV-B. The filters were replaced twice per week. UV lamps were placed 45 cm (sUV<sub>1</sub>) and 30 cm (sUV<sub>2</sub>) above the plant canopy to ensure the respective doses. UV-B intensity was measured with an ultraviolet intensity meter (UV-P Inc., San Gabriel, U.S.A.), and UV-B<sub>BE</sub> values was determined with a Spectro Power Meter (Scientech Inc, Boulder, CO, U.S.A.) normalized at 300 nm (Caldwell, 1971) to obtain  $UV-B_{BE}$  radiation.

#### PLANT SAMPLING AND GROWTH ANALYSIS

Plants were sampled randomly from the triplicate plots of each treatment for analysis of the parameters at 40, 70 and 100 days after transplantation (DAT). Six plants from each treatment were selected for measurement of growth characteristics and biomass. For determination of dry matter and its pattern of allocation in different plant parts, plant samples were oven-dried at 80°C for 24 h to constant weight. Prior to drying, the underground parts were washed thoroughly to remove dust/soil particles.

Leaf area was measured using a leaf area meter (LI-3000, LI-COR Inc., U.S.A.). A portable photosynthetic system (LI 6200, LI-COR Inc., U.S.A.) was used for measurement of photosynthesis (Pn), stomatal conductance (Cs), transpiration rate (E) and water use efficiency (WUE). Photosynthetic measurements were made with fully expanded leaves (third leaf from apex) in six plants from each treatment under ambient insolation, temperature and  $CO_2$  concentration, on clear days at noon; photosynthetic photon flux density (PPFD)

Plant age	Treatment	Total biomass (g/plant)	Plant height (cm)	Leaf area (cm²)	Leaf number/plant	Branch number/plant
40 DAT	$\begin{array}{c} C\\ sUV_1\\ sUV_2 \end{array}$	3.36±0.17⁵ 4.87±0.48ª 3.83±0.18ª⁵	44.96±4.69≈ 35.90±2.02 34.86±2.76♭	156.11±11.18ª 141.77±12.24ª 113.46±9.05b	5.66±0.57 <sup>b</sup> 9.66±0.25ª 7.00±0.7ª <sup>b</sup>	1.2±0.2 1.2±0.2 1.6±0.24
70 DAT	Control sUV1 sUV2	5.66±0.29 7.50±0.41ª 6.85±0.13ª	51.93±3.4ª 44.90±5.2⁵ 40.67±4.1⁵	386.25±31.25∝ 336.54±23.97∝ 201.55±12.95⁵	6.00±0.1⁵ 9.67±0.58ª 7.66±0.57⁵	1.8±0.27 2.4±0.30 2.1±0.29
100 DAT	Control sUV1 sUV2	16.03±0.34♭ 18.87±0.30ª 17.17±0.45♭	66.13±6.33∝ 55.80±4.12♭ 52.63±4.7♭	709.29±43.05∝ 662.65±51.77⁵ 426.72±35.19⁵	10.00±0.1⁵ 17.00±1.6≈ 14.67±1.2≈	2.0±0.32 2.8±0.2 2.4±0.24
ANOVA effects						
Plant age (A)		***	***	***	***	*
Treatment (T)		***	***	**	***	**
$A \times T$		*	ns	*	*	ns

TABLE 1. Effect of two excess UV-B doses on plant biomass, height, leaf area, leaf and branch number per plant in *Acorus calamus* L., by age

Values with different letters in the same column differ significantly at p < 0.05. Values are means ±SE. \*p<0.05, \*p<0.01, \*\*p<0.001, ns: Not significant.

was 1100–1200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. Growth indices – specific leaf area (SLA), leaf area ratio (LAR) and specific leaf weight (SLW) – were calculated with the formulae given by Hunt (1982).

#### PIGMENT CONTENT AND PAL ACTIVITY

Pigment content was measured in triplicate from leaf discs  $(1.5 \text{ cm}^2)$  from the same leaves used for photosynthesis measurement. For estimation of pigments, leaves were extracted in 80% acetone and absorbance of leaf extract was measured on a UV-vis. spectrophotometer (Systronics, model 117, India) at 480 and 510 nm for carotenoids and at 645 and 663 nm for chlorophyll estimation. The amounts of photosynthetic pigments were calculated with the formulae described by Maclachlan and Zalick (1963) for Chl a and b, and Duxbury and Yentsch (1956) for carotenoids. Flavonoid content was determined according to the method described by Flint et al. (1985). Flavonoids were extracted by homogenizing fresh leaf samples in acetic ethanol; the absorbance of the extract was measured at 260 to 320 nm and the resulting absorbance profile was plotted as described by Flint et al. (1985). Phenylalanine ammonia lyase (PAL) activity was assayed spectrophotometrically by measuring the amount of t-cinnamic acid formed at 290 nm according to the method developed by Subba Rao and Towers (1970). The reaction mixture contained L-phenylalanine (0.01 M, pH 8.7), tris HCl buffer (0.05 M, pH 8.8) and 0.1 ml enzyme extract in a final volume of 3 ml water. Enzyme activity was expressed as µmol t-cinnamic acid min<sup>-1</sup> g<sup>-1</sup> fresh weight, and specific activity was expressed as µmol t-cinnamic acid min<sup>-1</sup> mg<sup>-1</sup> protein.

#### STATISTICAL ANALYSIS

The significance of differences between the control and UV-B exposed plants was verified with Duncan's multiple range test (p<0.05). Multivariate analysis was applied to identify significant effects of plant age, sUV-B and their interactions. All statistical analyses employed SPSS ver. 10.0 (SPSS Inc.).

## RESULTS

The effects of sUV-B treatment on plant height and various growth traits are shown in Tables 1 and 2. Plant height and leaf area significantly decreased with increased sUV-B. At 100 DAT, plant height decreased by 15.6% ( $sUV_1$ ) and 20.4% ( $sUV_2$ ) and leaf area decreased by 20.7% (sUV<sub>1</sub>) and 39.9% $(sUV_2)$  versus the control (Tab. 1). Number of leaves per plant and number of branches per plant increased under sUV-B, with a greater increment at the lower dose, showing an increment of 61.2% (70 DAT) and 70% (100 DAT) in the number of leaves, and in the number of branches by 33.3% (70 DAT) and 40% (100 DAT) in sUV<sub>1</sub> plants versus their controls (Tab. 1). Multivariate analysis for morphological parameters showed significant variation of plant height, leaf area and number of leaves per plant due to plant age and treatment (Tab. 1). SLW increased under enhanced UV-B, with maximum increments of 151.3% (40 DAT) and 75.3% (100 DAT) in  $sUV_2$ exposed plants. SLA and LAR decreased significantly due to sUV-B treatment, with maximum reduction of 58.8% for SLA and 36.2% for LAR in sUV<sub>2</sub>-treated plants at 100 DAT (Tab. 2). Multivariate analysis for these growth traits also showed significant effects of plant age, sUV-B and their interactions (Tab. 2).

Plant age	Treatment	SLA (cm² g <sup>-1</sup> )	LAR (cm <sup>2</sup> g <sup>-1</sup> )	SLW (mg cm <sup>-2</sup> )
40 DAT	$\begin{array}{c} C\\ sUV_1\\ sUV_2 \end{array}$	447.00 ± 33.10 <sup>b</sup> 280.41 ± 29.55 <sup>b</sup> 186.95 ± 13.71 <sup>a</sup>	70.30 ±15.04 <sup>a</sup> 44.64 ±10.72 <sup>b</sup> 29.54 ± 5.55 <sup>b</sup>	1.87 ± 0.13° 3.69 ± 0.20° 5.47 ± 0.47ª
70 DAT	$\begin{array}{c} C \\ sUV_1 \\ sUV_2 \end{array}$	293.00±21.10° 139.00±10.80° 120.66± 9.00°	$46.22 \pm 3.41^{a}$ 29.47 ± 2.89 <sup>b</sup> 29.32 ± 2.17 <sup>b</sup>	$3.43 \pm 0.36^{b}$ $7.21 \pm 0.55^{a}$ $8.62 \pm 0.52^{a}$
100 DAT	$\begin{array}{c} C\\ sUV_1\\ sUV_2 \end{array}$	204.85 ± 17.00 <sup>b</sup> 135.64 ± 22.24 <sup>b</sup> 116.04 ± 26.72 <sup>b</sup>	44.26 ±3.64≈ 29.75 ±2.66⁵ 25.70 ±2.83⁵	4.94 ± 0.46 <sup>b</sup> 7.53 ± 0.31ª 8.66 ± 0.26ª
ANOVA effects				
Plant age (A)		***	**	***
Treatment (T)		***	***	***
$A \times T$		**	*	*

TABLE 2. Effect of two excess UV-B doses on specific leaf area (SLA), leaf area ratio (LAR) and specific leaf weight (SLW) of *Acorus calamus* L., by age

Values with different letters in the same column differ significantly at p < 0.05. Values are means  $\pm$  SE.

\* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

It is interesting to note that although root and shoot lengths decreased in sUV-B-exposed plants as compared to control plants, rhizome growth increased at the lower sUV-B dose, with a 22.5% increment at 100 DAT; at the higher dose rhizome growth was not affected (Fig. 1).

Biomass change versus the control was not significant in the  $sUV_2$  treatment; under the lower dose it increased (Tab. 1): the increment of total biomass per plant was 44.9% (40 DAT) and 17.7% (100 DAT) in  $sUV_1$ -exposed plants. Multivariate analysis showed significant differences in biomass due to plant age and sUV-B as well as their interaction (Tab. 1). Changes in biomass allocation to different plant parts of control and sUV-B-exposed plants are given in Figure 2.  $sUV_1$  exposure led to an increase in shoot biomass by 19.4%, root biomass by 11.3% and rhizome biomass by 27% at final age of sampling. Differences in biomass translocation between control and  $sUV_2$ -treated plants were not significant.

Even after long-term sUV-B exposure, visible symptoms of damage did not appear in plants grown under the lower dose. At the higher dose there was minor damage in the form of terminal and marginal chlorosis in older leaves. Total chlorophyll and the chlorophyll *a/b* ratio showed nonsignificant changes in the control and sUV-B-exposed plants at all sampling ages (p>0.05) (Fig. 3). Carotenoid content significantly increased in sUV-B treated plants, with increments of 60.1% at 70 DAT and 77.6% at 100 DAT in sUV<sub>1</sub>-exposed plants (Fig. 3). There were also significant differences in the absorbance patterns of flavonoid pigments recorded between 280 and 310 nm (Fig. 4). At 280 nm, the maximum observed increment of flavonoid content was 67.6% under  $sUV_1$ , followed by 39.4% in  $sUV_2$ -treated *Acorus* plants at 100 DAT. Phenylalanine ammonia lyase (PAL) also showed increased activity in sUV-B-treated leaves (Fig. 4). At 100 DAT, specific activity of PAL showed an increment of 47% in  $sUV_1$ -treated plants, and 25%. in the  $sUV_2$  treatment. Trends in PAL activity were well correlated with the increasing trend of flavonoid induction for both sUV-B treatments (Fig. 4).

The photosynthetic activity of *A. calamus* exhibited a positive response under  $sUV_1$  in this study: Pn increased by 26.4%, Cs by 30.5% and WUE by 80.7% in  $sUV_1$ -treated plants at 100 DAT (Fig. 5). Pn and Cs did not significantly vary in  $sUV_2$ -exposed plants versus their controls (Fig. 5). The increase of photosynthetic activity in  $sUV_1$ -exposed plants was well correlated with stimulation of assimilatory performance, resulting in increased biomass production. Transpiration rates initially declined in the sUV-B treatment but showed nonsignificant variation at later stages.

## DISCUSSION

In the present study, sUV-B exposure significantly reduced plant height and leaf area, and the number of branches and leaves increased, producing shorter and more compact plants. Inhibition of plant height was dose-dependent and was consistent with earlier studies on *Fagopyrum tataricum* (Yao and Liu, 2006) and *Gossypium hirsutum* (Gao et al., 2003). In *G. hirsutum* under UV-B exposure, Kakani et al. (2003b) reported a decrease in plant height by 16% (8 kJ m<sup>-2</sup> d<sup>-1</sup>) and 34% (16 kJ m<sup>-2</sup> d<sup>-1</sup>). Reduction of



**70 DAT** 

**40 DAT** 

80

60

40

20

0

60

40

20

0

60

40

20

0

Length (cm)



**Fig. 1.** Changes in growth of *Acorus calamus* exposed to UV-B radiation. Bars showing different letters differ significantly by Duncan's test at p < 0.005 (Mean  $\pm$  SE).

ZZZZZA SUV

Rhizome

Plant part

Leaf

sUV2

Root

C

**Fig. 2.** Changes in dry weight of *Acorus calamus* exposed to UV-B radiation. Bars showing different letters differ significantly by Duncan's test at p < 0.005 (Mean  $\pm$  SE).

plant height as a result of shortening of internodes has been reported (Zhao et al., 2003; Kakani et al., 2003a). Reduction of plant height and leaf area under UV-B stress has also been reported in, for example, Solanum tuberosum (Santos et al., 2004), Spirodela polyrhiza (Farooq et al., 2005) and Triticum aestivum (Agrawal et al., 2004). Hopkins et al. (2002) reported reduction of plant growth under UV-B stress as a result of alteration of the rate and duration of cell division and elongation, perhaps due to inhibition of indole acetic acid (IAA), a key regulator of plant growth. UV-B-induced oxidation of IAA may also be related to increased axillary branching. Increased leaf number and axillary branching such as we report under low-dose sUV-B are commonly observed plant morphogenetic responses under UV-B stress (Meijkamp et al.,

2001). Acorus calamus L. exposed to UV-B showed increased SLW and increased leaf thickness, which are considered important protective responses to UV-B radiation (Santos et al., 2004; Kakani et al., 2003a). Along with morphological changes in aboveground shoot parts, UV-B also affects patterns of root and rhizome development. Although root length decreased at the lower dose of sUV-B, rhizome size increased. Rhizome growth was promoted under lower-dose sUV-B; this is a point of interest in the present study, as it is a part of A. calamus containing the essential oil, having great medicinal value. Similar results showing UV-B-induced stimulation of the growth of belowground propagating organs have been reported in some plant species of a fen ecosystem in Tierra del Fuego, Argentina (Zaller et al., 2004).

100 DAT



**Fig. 3.** Effect of supplemental UV-B radiation on photosynthetic pigments content in *Acorus calamus* leaves. Bars showing different letters differ significantly by Duncan's test at p < 0.005 (Mean  $\pm$  SE).

Acorus calamus showed increased biomass production efficiency in the  $sUV_1$  treatment. Bassman et al. (2002) reported a similar positive response of biomass to UV-B in Douglas fir (Pseudotsuga menziesii). Petropoulou et al. (1995) also reported increased biomass under enhanced UV-B, with no marked cost for modification of maintenance and other metabolic processes. Kim et al. (1996) found no significant changes in biomass and pigment content in three UV-B-radiated rice cultivars. In the present study, the biomass allocation pattern showed a greater proportion allocated to belowground parts (rhizomes) after sUV-B treatment. Stimulated production of rhizome biomass at the lower sUV-B dose is one of our chief findings here.

Acorus calamus was able to maintain chlorophyll levels in both sUV-B exposure treatments. Since photosynthesis is dependent on the light-har-



**Fig. 4.** Effect of supplemental UV-B radiation on (a) flavonoid content and (b) PAL activity of *Acorus calamus* at 100 DAT (Mean  $\pm$  SE).

vesting complex of chlorophyll a, the absence of a significant reduction of chlorophyll should promote tolerance to UV-B stress (Greenberg et al., 1997). The increase in carotenoid content under sUV-B is an adaptive mechanism, acting as a quencher of reactive oxygen species and protecting sensitive targets (chlorophyll) from intense irradiation by screening excess excitation energy via the zeaxanthin-associated energy dissipation pool and the epoxidation state. Increased levels of flavonoids and PAL activity are considered important defense responses of UV-B-tolerant species (Wilson et al., 2001). Flavonoids, which act as a UV-B filter, also possess a capacity to scavenge free radicals, which might help protect UV-B-exposed plants. Up-regulation of genes of the phenylpropanoid pathway is a



**Fig. 5.** Effect of supplemental UV-B radiation on: (a) Stomatal conductance, (b) Net photosynthesis, (c) Water use efficiency of *Acorus calamus* at initial and final sampling ages, (d) Transpiration rate. Bars showing different letters differ significantly by Duncan's test at p < 0.005 (Mean  $\pm$  SE).

common response to UV-B, resulting in enhanced production of flavonoids due to increased PAL activity. It appears that the *Acorus* plants protected against photosynthetic damage from UV-B by producing higher amounts of carotenoids and flavonoids.

Photosynthesis is the most important metabolic process of plants, essential for production of biomass. Although sUV-B exposure resulted in decreased plant height and leaf area, it did not cause inhibition of net photosynthesis in A. calamus; the outcome was no significant change in biomass production. The increased biomass production of Acorus plants under  $sUV_1$  may be attributable to higher carbon assimilation capacity via the substantial increase in photosynthetic rates. Photosynthetic efficiency was lower under the higher than under the lower dose of UV-B irradiation, but the differences between the higher-dose treatment and the control plants were not significant. Some studies have reported higher accumulation of UV-B-absorbing compounds in the epidermal vacuolar region, minimizing the penetration of harmful UV-B radiation to photosensitive tissues (Tevini et al., 1991; Smith et al., 2000.). The lack of inhibition of photosynthetic activity under UV-B supplementation in our experiment accords with the results of earlier investigations (Ziska et al., 1993; Bassman et al., 2002). Bassman et al. (2002) reported increased net photosynthesis under moderate UV-B irradiance in *Pseudotsuga menziesii*; a higher dose was lethal. In our work the lower sUV-B dose boosted the photosynthetic rate through increased stomatal conductance. Stomatal conductance can play a major role in photosynthetic performance under stress conditions. Increased stomatal conductance under low UV-B supplementation could also enhance WUE. sUV-B radiation did not negatively affect the photosynthetic efficiency of the plants, so that total biomass was not reduced.

In fact, growing *A. calamus* under the lower UV-B dose might be advantageous in view of the substantial increase in its efficiency of production, especially production of rhizome biomass. Calabrese and Baldwin (2001) noted maximum stimulation of biological activities at low doses due to the nature of the hormetic dose-response phenomenon. *Acorus* improved its performance under low-dose sUV-B by optimizing the photosynthetic system and stomatal regulation, leading to better production efficiency. The beneficial effect of low doses (hormesis) is broadly discussed in biomedicine, especially in toxicology and radiation biology; Calabrese and Baldwin (2001) explained that overcompensation induced by hormesis is an adaptive response to low levels of stress or damage, yielding enhanced fitness for some physiological processes for finite periods. It results from modest overcompensation of disrupted homeostasis.

## CONCLUSION

Here we showed that the growth of *Acorus* plants was adversely affected under sUV-B exposure, with the response dependent on the dose. Maximum reduction of growth was noted under the higher dose of UV-B ( $sUV_2$ ). Higher flavonoid content due to increased PAL activity was found in plants exposed to the lower dose of UV-B ( $sUV_1$ ). Increased photosynthetic rates in  $sUV_1$ -treated plants led to higher accumulation of biomass. Since the medicinal value of *Acorus* plants is confined mainly to its rhizome, the increment in its growth at the lower dose of sUV-B is an important finding of this study.

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