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ORIGINAL ARTICLE

Drought and UV stress response in *Spilanthes acmella* Murr., (tooth-ache plant)

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Plants may activate similar defense systems to reduce cellular damages caused by different stress conditions. A wide range of biological changes in plants were attributed to elevated UV radiation (Caldwell et al., 2007). There are three potential targets for UV radiation in plant cells, the genetic system, the photosynthetic system and membrane lipids (Jansen et al., 1998). Enhanced UV radiation affects plant development; in particular biomass distribution and the reproduction stage (Kakani et al., 2003). In natural conditions, effects of UV radiation on plants are related to other environmental factors such as environmental stress (Caldwell et al., 2003). Reports on influence of UV radiation on photosynthesis are inconsistent due to differences in crops, UV dosages, and other environmental conditions (Kakani et al., 2003). Some plant species are unaffected by UV-B irradiation and several are apparently stimulated in their growth, but most species are sensitive and

damage results (Teramura, 1983). Coleman and Day (2004) reported that as the UV dose approached the ambient level, cotton and sorghum produced more branches or tillers, but with a smaller leaf area. Water stress or soil drought is an important restricting factor, which limits the productivity of many crops and affects both quality and quantity of the yield. Drought stress brings about a reduction in growth rate, stem elongation, leaf expansion and stomatal movements. It also affect various physiological and biochemical processes governing plant growth and productivity (Daie, 1997).

Plants developed a number of strategies to guard themselves against UV radiation, such as thicker and smaller leaves (Bornman et al., 1997), increased production of UV-absorbing compounds such as flavonoids, anthocyanins (Tevini et al., 1991; Hosseini, 2008), and higher amounts of reflective waxes (Rozema et al., 1997) and mechanisms of stress avoidance (e.g. accumulation of UV screens or stomatal responses) and of stress tolerance (e.g. DNA repair or synthesis of antioxidants). The accumulation of phenolic compounds is a key protective response of plants against UV radiation. Flavonoids are important plant phenolics which act as UV screens, antioxidants and energy-dissipating agents (Balakrishnan et al., 2005). The accumulation of flavonoids in the epidermis was shown to reduce epidermal transmittance of UV radiation and thus may provide some protection (Tevini et al., 1991). Some reports showed that carotenoids, anthocyanins, flavonoids and proline content increased significantly by decreasing ultraviolet wavelength (Balouchi et al., 2009; Mpoloka, 2008**)**, and low dose of UV-C radiation produced same response as UV-B exposure (Nasibi and Kalantari, 2005).

Drought can limit plant productivity even further, e.g. due to strong decreases in cell expansion and reduced photosynthesis (Reddy et al., 2004). Drought mostly affects accumulation of some organic compatible solutes such as sugars, betaines and proline, which adjusts the intercellular osmotic potential, is also early reaction of plants to water stress. The oxidative stress which caused metabolic damage in water stress, increases lipid per oxidation, resulting in greater membrane injury and pigment bleaching (Moller et al., 2007). Abdollah (2010) suggested that proline accumulation of plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism. Payam (2011) found that proline is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plants to avoid detrimental effects of water stress. The content of MDA (Malone dialdehyde) has been considered an indicator of oxidative damage (Moller et al., 2007; Magdalena et al., 2010). MDA is considered as a suitable marker for membrane lipid peroxidation.

Spilanthes acmella Murr. - “toothache plant” (Asteraceae) is known as one of the important medicinal plant and it is very sensitive to drought since it favors moist soils to flourish. It has a long history of use as a folklore remedy, e.g. for toothache, rheumatism and fever (Agarkar, 1991). The plant has found applications in pharmaceuticals as an anti toothache formulation for pain relief, swelling and gum infections (John, 2001). The plant extract was used for stimulating, reorganizing and strengthening the collagen network in anti-age applications, e.g. in anti wrinkle cream formulations (Sharma et al., 2010). A decoction of the plant can be taken internally as a diuretic and able to resolve stones in the bladder, while a decoction of the roots

can be used as a purgative. It is also used as a preventive medicine for scurvy and stimulates digestion (Verma et al., 1993). Besides these medicinal uses, the flower heads have been used as a spice for appetizers by the Japanese and its extract was used as a flavouring material for dentifrices and gum (John, 2001). Now nothing seems to be published on the effect of stresses in Spilanthes acmella. Therefore, the objective of this research was to investigate the changes in photosynthetic pigments and other physiological and biochemical traits of S. acmella exposed to ultraviolet radiation and water stress.

MATERIALS AND METHODS

Plant Material

Spilanthes acmella (toothache plant) collected from Karakulam (Thiruvananthapuram) was used for this study. The plant was grown in Botany Department Garden, Kariavattom, Thiruvananthapuram (Kerala). The plant materials selected for the present study were the clones of a single mature plant, replicated by stem cuttings. Crop management was done according to the recommended agronomic practices.

Experimental design

Plants were grown in separate pots. Three-week-old plants were exposed to water withdrawal and to daily irradiation by UV radiation. The plants were then exposed to UV-C for 60 min. from 15cm distance of UV source (approx. I.5 kJ m^{-2}). Plants unexposed to UV-C were used as control. All measurements were made after 3days and 5days after UV treatments. For drought treatment, the pots were kept without giving water for 7 days. Well watered plants were kept as control. All studies for drought treatment were done after 7 days.

Morphological characteristics

The height and leaf numbers of all plants were measured before and after stress. The leaf area was determined using graphical method. Leaf colour, leaf size, curling of leaves, appearance of leaf surface was compared before and after treatment.

Estimation of relative water content

Relative water content was determined using the method of Barrs and Weatherly (1962). Relative water content of fully expanded last leaves was estimated. Leaf material was weighed (0.2 g) to determine fresh weight and placed in double-distilled water for 4 h and then turgid weight was recorded. Finally, the samples were dried in an oven at 65°C for 48 h and the dry weights were recorded and relative water content was calculated.

Anatomical characteristics

Anatomical parameters, including presence of trichomes and epidermal thickening were determined on stem portions collected from the control and treated plants. Transverse sections of stem were taken and stained with saffranine and mounted in 50% glycerol. Digital images were obtained from wet mounts with a digital camera (DP11, Olympus, Tokyo; Japan) attached to the microscope.

Biochemical study

The fresh leaf materials collected were immediately used for the extraction and assay according to the appropriate methods given below.

Determination of photosynthetic pigments

Pigment content was measured from leaf discs. For estimation of pigments, 0.1g of leaf material was ground in 2ml acetone (80%), extract was centrifuged at 2700g for 10 minutes. The absorbance of the supernatant was measured on a UV-vis. spectrophotometer (Pharmaspec. UV-1700,

Shimadzu) at 480 nm for carotenoids and at 645 and 663 nm for chlorophyll estimation. The amounts of photosynthetic pigments were calculated with the formulae described by Sadhasivam and Manickam (2003).

Determination of UV absorbing pigments

Anthocyanin assay. Anthocyanin content was estimated according to the method of Fulki and Francis (1968). Leaf samples (0.1gm) were homogenized in a mortar and pestle with10 ml 1% HCl-methanol solvent (1: 99, v: v). The homogenate was centrifuged at 18000g for 10 min at 4°C, and the supernatant was filtered through Whatman no:1 to remove particulate matter and was stored in darkness at room temperature for 24 h. The amount of anthocyanin was determined from the absorbance at 550 nm. Anthocyanin content was expressed as mg/g fr. weight and the concentration of anthocyanin was calculated.

Flavonoid assay. Flavonoid content was determined according to the method described by Bohan et al (1994). Leaf samples (8gm) were homogenized in a mortar and pestle with 80% aqueous methanol at room temperature. The homogenate was filtered through Whatman no: 1 to remove particulate matter. The filtrate was incubated in a water bath for 10 min at 80°C and then allowed to cool to room temperature and weighed.

Measurements of free proline concentration

Proline assay. Proline content was determined according to the method of Bates et al. (1973).Samples of leaves (0.2 g) were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3% w/v), and then the homogenate was centrifuged at 18000g for 15 min. Supernatant was then put into a test tube into which 2 ml glacial acetic acid and 2 ml freshly prepared acid ninhydrin solution were added. Tubes were incubated in a water bath for 1 h at 100°C, and then allowed to cool to room temperature. Four ml of toluene were added and mixed on a vortex mixer for 30 s. After, the toluene phase was carefully pipetted out into a glass test tube, and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was calculated from a proline standard curve and was expressed as µg/g fresh - weight.

Determinations of antioxidant enzymes

Catalase assay. Catalase activity measured by the method of Sadasivam and Manickam (2003). Collected leaves (1g) were homogenized in a mortar and pestle with 20ml sodium phosphate buffer (pH 7.0) and centrifuged at 4^{o}C for 10 minutes at 10000 xg. 0.04 ml of the enzyme extract was added to the reaction mixture containing 1ml of 0.01 M H_2O_2, 3 ml sodium phosphate buffer (pH 6.8). The CAT activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm against control cuvette containing enzyme solution as in the experimental cuvette, but containing H_2O_2 free phosphate buffer.

Peroxidase assay. Peroxidase activity measured by the method of Sadasivam and Manickam (2003). Leaves (1 g) were homogenized in chilled extraction medium containing 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, and 1% (w/v) PVP. The reaction mixture of a total volume of 3 ml consisted of 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM Guaiacol, 2.5 mM H_2O_2 and 0.1 ml enzyme extract. The H_2O_2-dependent oxidation was followed by a decrease in the absorbance at 290 nm. Noted the time required in minutes (Δt) increasing the absorbance by 0.1 and enzyme activity was calculated.

Estimation of thiobarbituric acid substances.

For estimation of Thiobarbituric acid, of the leaf tissue (0.2g) were homogenized in 10ml of 0.1% trichloroacetic acid (TCA). After centrifugation, 1ml of the supernatant was vortexed with 4ml of 20% TCA containing 0.5% 2-thiobarbituric acid (TBA), and heated for 30 minutes at 95°C.The samples were cooled on ice for 5min and centrifuged for 10minutes at 10000g. The non-specific absorbance of supernatant at 600nm was subtracted from the maximum absorbance at 532nm for the MDA measurement (Heath and Packer, 1969) and at 455nm for other aldehydes. For the MDA and aldehydes calculation, an extinction coefficient [E] of 1.56 x 10^5 $M^{-1}cm^{-1}$ was used at 532nm for MDA and an [E] of 0.457 x 105 $M^{-1}cm^{-1}$ was used at 455nm as the average of the E obtained for five other aldehydes (propanal, butanal, hexanal, heptanal and propanal-dimethyl acetal). MDA and aldehydes in the leaves were analyzed following Carmark and Horst (1991).

Statistical analysis

Quantitive changes of different parameters were analyzed through analysis of variance (ANOVA). All values were means of five replicates per treatment. Statistical significance was calculated at $P< 0.05$ according to Duncan's multiple range tests. All the statistical tests were performed using SPSS (Statistical Package for the Social Science) software (SPSS, version 7.5, Chicago, IL, USA).

RESULTS

Effect of UV and drought stress on growth pattern and morphological characters

The effects of the stresses on morphological characters and growth of *S. acmella* measured are documented. In general, the growth was retarded by both the stresses tested. However, the inhibited effect was different for different stresses.

Reduction of shoot length and leaf area was noticed during drought and UV treatment. But it was not significant. After the exposure to UV shoot height showed 2.69% decrease in T1 and 3.69% in T2 as compared to control. Lowermost shoots were observed after drought treatment (D), 6.82 % decrease observed compare to that of control. Leaf numbers were not affected by UV radiation but they were significantly reduced by drought. Adverse effect of UV radiation on leaf area was also determined during prolonged exposure. No difference was noticed in the leaf area in the 3 day exposure (T1), however 7% decrease in leaf area were observed in the 5 day exposure (T2). In drought, only 1% decrease was observed as compared to control.

Morphometric changes such as curling of leaves and shiny surface due to waxy coatings were noticed in plants grown under UV radiation. After one day of treatment there was no noticeable change except the colouration of leaves. The colour of leaves changed to dark green. After 3 days exposure the colour of the mature leaves were turned to brown. The older leaves showed senescence after 5days exposure and yellowing, wrinkling and curling occurred in young as well as mature leaves. Observations were made every day after UV treatment in field condition. The first day after treatment the yellow leaves turned to brown and withered. New leaves were developed but it showed reduced size and wrinkled appearance and the pest attack was severe in treated plant than in the control.

There were reductions in the plant height and leaf area when exposed to drought. But it was not significant. Yellowing was observed in entire leaves. All mature leaves showed senescence. Severely

drought affected plants recovered after well irrigation and showed normal growth within nine days. Curling and waxy coating of leaves were not observed.

Effect of UV and drought stress on anatomical characteristics

Plants from both treatments exhibited similar leaf anatomy. Drought treatment had no significant effect on thickness of leaves, adaxial or abaxial epidermis, or palisade or spongy mesophyll. No treatment differences were observed in the extent of inter cellular air spaces present in the spongy mesophyll. But UV treated plants exhibited epidermal thickening (thick cuticle) and trichomes. Histochemical localization of proteins, starch, phenols and flavonoids showed its presence in both the treatment as control.

Effect of UV and drought stress on relative water content

Relative water content markedly increased in UV treated plants than the control. In drought treatment, the reduction of RWC was significant (44.93%) in comparison with the control. T1 plants showed 68.05% and T2 plants exhibited 77.35% of RWC, whereas in control RWC was 62.89% (Graph: I).

Effect of UV and drought stress on chlorophyll

In the present study the photosynthetic pigments such as chlorophyll *a* and *b* were analyzed both in the control and treated plants. It is documented in Table 3. In drought treated plants chlorophyll *a*, chlorophyll *b* and total chlorophyll contents were considerably decreased, as compared with the control plant. It was 16.4% in chlorophyll *a,* 45.7% in chlorophyll *b* and 31.35% in total chlorophyll. In UV treatment the chlorophyll *a,* chlorophyll *b* and total chlorophyll contents were decreased as 44.5%, 55.5% and 41.90% respectively in T1 where as 59 %, 63% and 68.33% respectively in T2 (Graph: II).

Effect of UV and drought stress on carotenoids, flavonoids and anthocyanins

Effect of UV and drought stress on carotenoids, flavonoids and anthocyanins is documented in Table 4. In UV treatment, the carotenoid concentration increases when the extent of treatment increases. Water-stressed plants showed 17.13% increase whereas T1 and T2 plants showed 39.3% and 60.2% increase respectively when compared to that of control (Graph: III).

UV absorbing compound, flavonoids and anthocyanins were analyzed in both treatment. In UV treated plants flavonoids and anthocyanins increased abundantly. In drought plants both the compounds decreased very much as compared with the control plant. Drought plants showed 29.2% decrease in flavonoids and 57.9% decrease in anthocyanins. Flavonoid concentration was increased 180% in T1 plants, while in T2 it was 270%. Anthocyanin concentration was also increased in T1 (124.5%) and in T2 (129.1%) plants (Graph: IV).

Effect of drought and UV stress on proline and MDA content

Proline content showed high increase in drought plants than UV plants. Proline content in UV treatment showed gradual increase with increases the extent of treatment. T1 plants showed 290.43% increase and T2 plants showed 376.51% increase in proline content as compared to that of control. Drought plants exhibited 595.06 % increase (Graph: V).

MDA accumulations were more significant in drought treatment than in UV treatment. At the

end of the drought experiment, compared with the well-watered plants (control), the increment of the MDA concentration in the water-stressed plants were 290%, whereas they were 71% and 215% in T1 and T2 plants respectively (Graph: VI).

Enzymatic assay

Antioxidant enzymes such as catalase and peroxidase, involved in the protection against membrane damage, were measured for drought and UV treatments. It is given in table 6.

Catalase activity

During drought condition the catalase activity decreased as compared with the control plant. Drought plants showed 5.31 units/L activity, while well-watered plants showed 8.90 units/L. UV treated plants showed increase in the catalase activity than control plants. The T2 plants showed more activity (11.47 units/ L) than T1 (9.32 units/ L) plants.

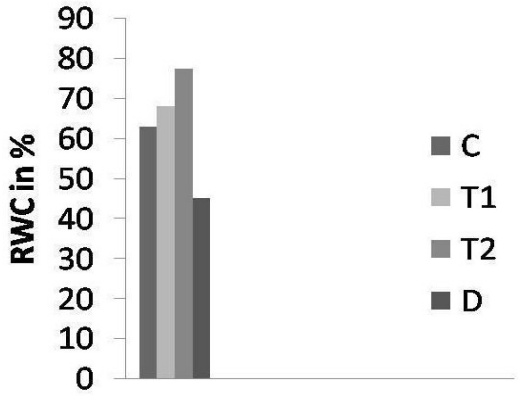
Peroxidase activity

Drought treated plants showed increase in the peroxidase activity than control plant. It showed 16.36 units/L activity while the control plant showed 9.52 units/L activity. UV treated plants also showed increase in peroxidase activity, T1 and T2 plants showed 14.89 units /L and 17.68units/L respectively. Therefore high activity of MDA in this study was recorded in T2 plants compared to T1 and drought.

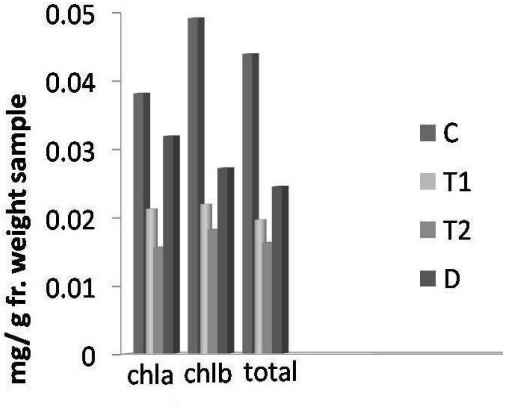
Statistical analysis

Statistical analysis of eleven variables showed that all the variables except RWC, shoot length and leaf area were significant.

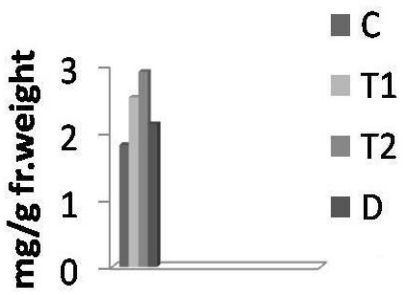
Graph I: Effect of UV and drought stress on relative water content



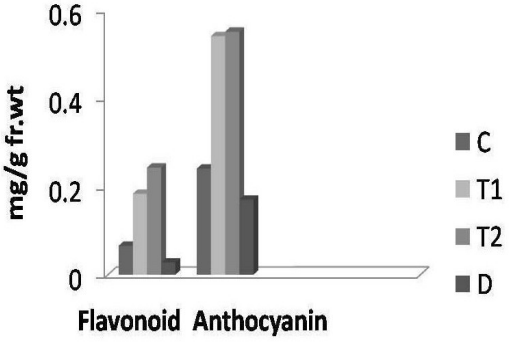
Graph II: Effect of UV and drought stress on chlorophyll content

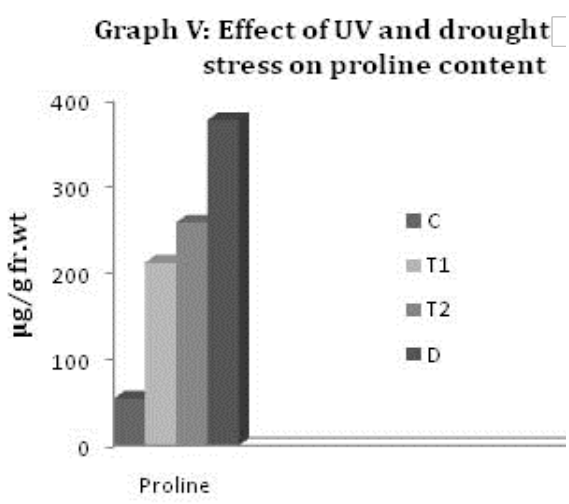


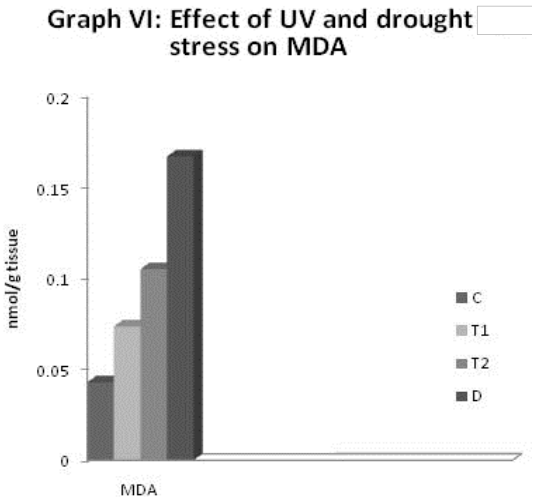
Graph III: Effect of UV and drought stress on carotenoid content



Graph IV: Effect of UV and drought stress on Flavonoid and Anthocyanin







DISCUSSION

Phenotypic plasticity in response to stressful environmental conditions has been recognized in a variety of species (Waring, 1991). Ultraviolet treatment of *Spilanthes acmella* showed some significant effects than drought on the main growth parameters. Other studies also show significant UV radiation effect on barley growth parameters including stem height, sprout count, leaf area and biomass (Correia et al., 1999; Nasser, 2001). Plant sensitivity to UV exposure might be determined by direct damage to cell structural and functional elements or by ineffective acclimatization process (Smith et al., 2000). UV-B radiation may induce leaf differentiation and senescence processes via modification of leaf structure (Kakani et al., 2003, 2004). Nevertheless, other authors (Liu et al., 1995; Schmitz-Hoerner and Weissenbock, 2003; Valkama et al., 2003) show that biomass or photosynthetic pigment content does not change under the exposure to UV-B radiation or such variation is insignificant. Plants are capable to accommodate to certain UV radiation as well as to light intensity, though tolerance range are determined by plant species, age, duration of exposure and other factors. If the UV-B dosage exceeds the limits of tolerance, plant leaf anatomy is changed and biomass is decreased (Coleman and Day, 2004; Kakani et al., 2003; Zhao et al., 2003). However in this study, no significant difference in the internal structure of leaf and stem showed that low dose of UV-C radiation was not very detrimental in *Spilanthes acmella*.

Hakala et al., (2002) determined sensitivity of various agricultural plant species including barley, wheat, oat, clove and potato to exposure to UV-B radiation and found no significant variation of biomass accumulation or yield. In the present study, UV-C radiation had no significant effects on leaf area and leaf thickness but showed some variations in appearance such as wrinkling and curling of leaves. Many reports are shown that the plant height response to UV radiation (Sullivan and Teramura, 1988). For example, decreased plant height often occurs in conjunction with increased stem diameter and self-shading by foliage, which reduces heat load at the base of the seedling and minimizes cellular damage that occurs at high surface soil temperatures. This research revealed that growth pattern of S. *acmella* was not very sensitive during UV radiation, however it showed

reduced leaf surface and shoot growth after treatment. Growth arrest can be considered as a possibility to preserve carbohydrates for sustained metabolism, prolonged energy supply, and for better recovery after stress relief.

In the present study, reduced growth and yellowing of leaves were observed in water-stressed plants. Severely water-stressed plants recovered after well irrigation and showed normal growth within nine days. Reports show that in soybean, the stem length was decreased underwater deficit conditions (Specht et al., 2001), and the plant height was reduced up to 25% in water stressed citrus seedlings (Wu et al., 2008). Stem length was significantly affected under water stress in potato, *Abelmoschus esculentus* (Sankar et al., 2007, 2008); *Vigna unguiculata* (Manivannan et al., 2007); soybean (Zhang et al., 2004) and parsley (*Petroselinum crispum*) (Petropoulos et al., 2008). Many reports show that water deficit stress mostly reduced leaf growth and in turn the leaf areas in many species of plant like *Populus* (Wullschleger et al., 2005), soybean (Zhang et al., 2004) and many other species (Farooq et al., 2009). The leaf growth was more sensitive to water stress in wheat than in maize (Sacks et al., 1997); *Vigna unguiculata* (Manivannan et al., 2007) and sunflower (Manivannan et al., 2007 and 2008). The inhibition of shoot growth during water deficit is thought to contribute to solute accumulation and thus eventually to osmotic adjustment (Anjum et al., 2011). In addition to that water stress greatly suppresses cell expansion and cell growth due to the low turgor pressure. Osmotic regulation can enable the maintenance of cell turgor for survival or to assist plant growth under severe drought conditions (Shao et al., 2008). Bhatt and Rao (2005) reported that the reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence in *A. esculentus* under water stress.

Relative water content (RWC) is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC related to water uptake by the roots as well as water loss by transpiration. A decrease in the relative water content in response to drought stress has been noted in wide variety of plants as reported by Balouchi et al., (2009) that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. The insignificant changes in RWC after UV-C treatments in this present study tend to indicate that the reduction, or the block, of growth did not involve the water content, which is the main cause of reduced growth in drought-treated plants. The slight increase in RWC of the plants exposed to UV-C could be interpreted as an increased plant protection from UV damage, because leaf thickening can reduce UV penetration. In addition to that the increase of RWC by UV treatment can be related to the induction of osmolytes or stress proteins faster than those induced by drought stress.

Photosynthesis is very important process in plants, is based on chlorophylls' system and, if such system is altered by UV radiation, chlorophyll decrease is hindered; hence, such feature might be used to determine UV sensitive plants. Accordingly, plants, which are able to keep chlorophylls' system unchanged, are far more resistant to UV radiation (Smith et al., 2000). During this study, amount of chlorophyll *a* significantly decreased if the exposure period increased. Amount of chlorophyll *b* was not also stable and decreased during the experiment.

Some authors have stated that content of chlorophyll *a* remains unchanged under the exposure to UV-B, while amount of chlorophyll *b* decreases (Barsig and Malz, 2000). However, the present study revealed that in *Spilanthes acmella*, chlorophyll *a* and chlorophyll *b* were more sensitive to UV-C radiation. Such variation could be based on the injury of thylakoid lumen, where the center of light harvesting system – chlorophyll *a* – is being damaged and disintegrates (Rengel et al., 1989). Decrease of chlorophylls' *a* to *b* ratio under exposure to UV radiation was also shown by other authors (Smith et al., 2000). Some reports showed that increase UV-B and UV-C irradiance also caused the reduction of the contents of chlorophyll a, b and (a + b) of pepper leaves (Mahdavian et al., 2008). The reduction of the chlorophyll content has a negative effect on plant photosynthetic efficiency. Since it has been reported that photosynthesis is dependent on the light harvesting properties of the chlorophylls (Balakrishnan et al., 2005). UV induced reduction in chlorophyll may be expected to result in lower levels of biomass accumulation, and hence be a useful indicator of UV sensitivity (Smith et al., 2000).

In general, UV radiation decreases the chlorophyll content at larger extent, since the chloroplast is the first organelle to show injury response when irradiated with UV radiation (Balakrishnan et al., 2005). Reduction in chlorophyll *a* and chlorophyll *b* contents might be due to inhibition of biosynthesis or due to degradation of chlorophyll and their precursors (Teramura, 1983). In the present study, gradual decrease of total chlorophyll content of the leaves under supplemental UV treatment might be due to that UV radiation may induce non-enzymatic photooxygenation of chlorophylls resulting in accumulation of these pigments as oxygenated forms. Another explanation linked with the decline in chlorophyll level might be due to inhibition of *cab* gene, which codes for chlorophyll protein (Balakrishnan et al., 2005).

Carotenoids are the main protective agents dissipating excess energy and protecting photoreaction center from auto-oxidation (Yamamoto and Bassi, 1996). The reduction in carotenoid content may result either from inhibition of synthesis or from breakdown of the pigments or their precursors (Yamamoto and Bassi, 1996). Since, the carotenoids are involved in the light harvesting and protection of chlorophylls from photoxidative destruction, any reduction in carotenoid could have serious consequences of chlorophyll pigments (Teramura, 1983). Some studies have shown that carotenoids serve a protective function against UV-B (Jaleel et al., 2008) and UV-C (Campos et al., 1991) radiation. The efficacy of carotenoids in protecting the photosystems is likely due to their function as efficient quenchers of high energy short wave radiation. The mechanism by which this is accomplished was first proposed to involve a photochemical state change of singlet oxygen to triplet form by interaction with carotenoids, removing the potentially dangerous oxygen radicals produced in photo oxidative processes (Krinsky, 1979). Present study revealed that content of carotenoids increased during first three days of exposure, and showed higher value at the fifth day of exposure. Thus, it could be suggested that *Spilanthes acmella* is very responsive and hardly adapt to the increased UV radiation. The same results were observed in drought plants but percentage of increase was not significant as compared to control. Water stress, among other

changes, has the ability to reduce the concentrations of chlorophylls and carotenoids (Havaux, 1998; Kiani et al., 2008), primarily with the production of ROS in the thylakoids (Niyogi, 1999; Reddy et al., 2004).

In the present study anthocyanin concentration was significantly increased in UV radiation treatment, when compared to control. Ambasht and Agrawal (1998) observed high increase in the anthocyanin content in maize. A 48 hours continuous irradiation of UV-B radiation increased the anthocyanin at four fold level in *Vigna* (Kulandaivelu, 1989). UV induced accumulation of anthocyanin protects the photosynthetic apparatus from the damaging effects of UV radiation. Flavonoids are ubiquitous plant secondary products that are best known as the characteristics red, blue and purple pigments of plant tissues (Balakrishnan et al., 2005) These compounds serve essential functions in plant reproduction by recruiting pollinators and seed dispersers. They are also responsible for the beautiful display of fall colour in many plant species, which has recently been suggested to protect leaf cells from photo-oxidative damage (Li et al., 2008). The first direct evidence in support of a role for flavonoids in UV protection came from experiments with *Arabidopsis* mutants, which showed that lesions in chalcone synthase (CHS) or chalcone isomerase (CHI) resulted in UV-hypersentive phenotypes. The flavonoids reduce the damage from UV radiation because they act as UV filters, reducing the penetration of potentially damaging UV radiation (Mahdavian et al., 2008). Similar to anthocyanin, flavonoid concentration was also increased in the present study after five days of treatment of UV and it was very high as compared to anthocyanin concentration. According to Tevini et al. (1991) flavonoid accumulation is regarded as a protective mechanism in higher plants to provide against UV radiation. Therefore the present study showed increase of anthocyanin and flavonoid content merely in UV treatment not in drought treatment. Hence, it is suggested that the UV treated plants may activate a defence mechanism against UV damage by increasing flavonoid and anthocyanin.

Prochazkova et al., (2001) reported that flavonoid concentration can reduce the UV-B penetration and protect the photosynthetic apparatus up to some extent, but it depends upon a threshold level which may vary in different species. However, there is also evidence that flavonoids may function in plants to screen harmful radiation, bind phytotoxins and help to regulate the stress response by controlling auxin transport (Mahdavian et al., 2008). Accumulation of anthocyanins and other UV-absorbing compounds, flavonoids and total phenols, after UV irradiation has been reported (Caldwell et al., 2007; Balakumar et al., 1993). They may act in the leaf as solar screens by absorbing UV before it reaches UV-sensitive targets such as chloroplasts and other organelles. However an increase of anthocyanins and flavonoids after UV irradiation was observed in the present study but a decrease of chlorophyll *a* and *b* suggests that an increase of such substances seems to be insufficient to act as a UV screen, and chloroplasts were damaged. The reduction in the UV damaging effect by UV absorbing substances is of course, a balance between the *de novo* synthesis of absorbing compounds and the energy of UV that reaches the leaves. In the present experiments a high intensity of UV-C light was applied, so the increase of anthocyanins and phenols was not enough to absorb the UV radiation that can reach the cell organelles and cause damage.

Most physiological stresses lead to disturbance in plant metabolism and cause oxidative injuries by enhancing the production of reactive oxygen species. On the other hand, plant resistance to stress factors is associated with their antioxidant capacity, and the increased levels of the antioxidant constituents may prevent stress damage. Additionally, Beggs, (1985) have proposed that when growth is restricted by some stress factor, other repair mechanisms such as photoreactivation, excision repair, quenching and free radical scavenging could be activated in order to alleviate the stress and prevent the damage before it becomes lethal. In the present study *S. acmella* leaves responded to the UV-C treatment by increasing significantly their flavonoid and anthocyanin contents, which presumably offers protection from the high UV-C level. The flavonoids play many defensive roles in plants, and interception of UV by epidermal flavonoids often proposed as an adaptive mechanism preventing UV from reaching the mesophyll and affecting photosynthesis (Liu et al., 1995). Thus, the *S. acmella* may activated a defence mechanism against UV damages by increasing non-photosynthetic pigments. The involvement of flavonols in the UV response has been reported for several plant species, including legume such as soybeans (*Glycine max*) (Middleton and Teramura, 1993). However, the antioxidant function of flavonoids is complex and depends on a variety of factors, including compartmentalization, redox potential, glycosylation and hydroxylation (Bors et al., 1995; Rice et al., 1996). This complexity therefore also needs to be taken into account in the consideration of possible antioxidant functions for increased flavonoid levels under UV treatment.

Proline was the stress marker measured that was increased primarily by the drought stress only. This is a typical plant stress response, well-described in water and salt stresses (Najaphy et al., 2010). Proline is known to be involved in alleviating cytosolic acidosis associated with several stresses (Smirnoff and Cumbes, 1989). The removal of excess H^+ occurring as a result of proline synthesis may have a positive effect on reduction of the UV induced damage. As one of the end products of lipid peroxidation, the MDA content reflects the degree of the peroxidation of membrane lipids (Taulavuori et al. 2001). H_2O_2 as a reactive oxygen species (ROS) damages the membrane lipids, and induces protein denaturation and DNA mutation (Bowler et al. 1992). The MDA contents significantly increased in drought stress, but MDA possessed not much effects in UV stress. The significant increase of MDA contents in the present study, suggested that drought stress caused oxidative damages. Similar results are reported in olive trees (Sofo et al. 2004), sunflower (Bailly et al. 1996) and *Coffea arabica* (Queiroz et al. 1998).

Oxidative stress in plants is mitigated by the activation of antioxidant defences, including antioxidant enzymes such as peroxidase (PEX), polyphenol oxidase, catalase (CAT), ascorbate peroxidase (AP), and glutathione reductase (GR). Oxidative stress is accompanied by the synthesis of hydrogen peroxide, which is normally detoxified by CAT activity in the peroxisomes and by AP in the cytosol, mitochondria, and chloroplasts. Peroxidase activity is also an important component of the antioxidant stress system for scavenging H_2O_2. However, catalase changes H_2O_2 into O_2, whereas peroxidase decomposes H_2O_2 by oxidation of co-substances and to promote the utilization of phenolic compounds as co-substrates (Gaspar et al., 2002). Gaspar et al. (2002) stated that increased

basic peroxidase activity in response to stress decreases the indole acetic acid concentration and promotes acidic peroxidase synthesis. Activation of antioxidant enzymes by UV-B has earlier been observed in *Arabidopsis thaliana*, wheat (Sharma et al., 2010), and cucumber. Varying responses in antioxidants under UV exposure have been reported, depending on species and intensity of radiation (Dai et al., 1997).

In this study, it was observed that activities of the two key antioxidant enzymes CAT and PEX varied following all the treatments compared to the control plants, but their expression patterns were different for different stress conditions. CAT expression pattern, however, was increased in UV and decreased in drought treatment. Consequently, CAT activity was greater in the first dose UV radiation (T1) than in the second (T2) at the end of the treatment. Similarly, PEX activity increased following the stresses of UV and drought, but the enhancement rate was lower in the first UV dose stress than in the second UV stress. The same trend was also reflected in the peroxidase gel. Therefore, different levels of H_2O_2 under all stresses, particularly higher H_2O_2 content in the higher UV dose than in the other two may be due to the differential expressions of activities of those enzymes following the stress. This result further proves previous observations that cellular H_2O_2 concentration is the result of the balance between its production and utilization (Bowler et al., 1992).

CONCLUSION

The morphological and biochemical parameters measured indicate that UV light has stronger stress effects than drought in *S.acmella*. Decrease of amounts of photosynthetic pigments indicates *S.acmella* is sensitive to UV radiation. Accumulation of anthocyanin and flavonoids pigments could act as solar screens by absorbing UV radiation up to some extent and protect the chloroplast from UV induced damage but it was insufficient. This is evidenced from low chlorophyll content after UV treatment. Indirect evidence from CAT and PEX activity suggests that UV exposure generates free radicals. In the present study, a marked increase in proline in drought and UV treatment represents adaptive responses against oxidative damage induced by stresses. Proline increased primarily in drought-stressed plants. Proline may be the drought-induced factor which has a protective role in response to UV. By considering to obtained results in this study it can be concluded that UV radiation is harmful to *Spilanthes acmella*. Further experiments are necessary for better understanding of the exact mechanism on the plant's response to UV and drought stress.

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