

PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF UV-RADIATION AND SODIUM DODECYL SULPHATE (SDS) MUTAGENS ON GROWTH AND METABOLISM OF GARLIC PLANTS

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ABSTRACT

The effects of enhanced UV_A (320-380), UV_C (280nm) and SDS, as mutagenic substances, on growth parameters and certain metabolic changes, during vegetative and flowering growth stages of garlic (*Allium sativum* var. Seds 40) were investigated. Root length, shoot length, fresh mass, dry mass accumulation and leaf area in garlic, treated with UV- radiation (A and C), and SDS (0.1 M and 0.3 M) as mutagenic substances, throughout the entire period of the experiment, showed significant variable changes below the control levels. Photosynthetic pigments (chl a, chl b, carotenoids, total chl a+b, and total pigment) contents of the variously treated garlic plants showed significant changes as compared with control plants throughout the duration of the experiment. The ratio of chl a / b showed variable changes in UV- and SDS- treated plants in relation to control. UV- absorbing compounds (total phenolic compounds and anthocyanins contents) of the UV- and SDS- treated garlic plants, showed significant increase above the control levels during vegetative and flowering growth stages. As compared with control nucleic acid levels, nucleic acid contents (DNA and RNA) of UV- and SDS- treated plants, at vegetative and flowering growth stages, showed significant decrease.

Keywords: UV_A and UV_C radiation, SDS, garlic, photosynthetic pigments, total phenolic compounds and anthocyanins, nucleic acids (DNA and RNA).

INTRODUCTION

Several physiological and biochemical constituents in plant cells undergo drastic changes as a result of exposure of the plants to supplementary UV radiation or treatment of plant tissue with chemical mutagenic substances (Bronman and Teramura, 1993). These changes occur at relatively high doses of UV radiation whereas other effects and responses are manifested at a dose of about one magnitude lower (Strid *et al.*, 1997). In *Pisum sativum*, these low dose effects include increased ion permeability of the thylakoid membrane and alterations in the mRNA transcript levels of photosynthetic and defensive protein components (Kalbin *et al.*, 1997).

Comparable amounts of genetic variation were induced by chemical mutagenic material (EMS), gamma rays and UV- radiation, but larger responses to selection and realized heritabilities followed EMS treatment of *Arabidopsis thaliana* plants. The most extreme mutants for latenes were selected after EMS treatment and for plant weight after EMS and radiation treatment (Brock, 2007). These results support the hypothesis that mutagenic treatment by chemical mutagens or physical mutagens (UV- radiation), gives

rise to an increase in variance for quantitatively inherited characters which can be utilized by selection.

Numerous studies have shown that exposure of plants to mutagenic materials, either chemical or physical can result in a wide variety of morphological alterations in higher plants (Burnes *et al.*, 2005). These morphological changes can be observed under controlled environmental conditions in growth chambers or greenhouse, where UV-emitting lamps provide the sole source of UV-radiation (e.g. reduced leaf area and shoot height), whole plant changes in morphology are the result of an inhibition in the elongation or expansion of individual organs (leaves and stems).

In addition it has been suggested that exposure to UV- radiation reduces plant growth vigor, chlorophyll contents, carotenoids, total protein content, nucleic acid content and increases the level of phenolic compounds and anthocyanins (Musil, 1996; Abdel-Aziz, 2008). Ultraviolet light inhibited the growth in four wheat cultivars (*Triticum aestivum* L.) and increased phenolic compounds and proline contents which were thought to protect cells against damage (Demir, 2000). Anthocyanins accumulate in young, expanding foliage of various plant species in response to UV- radiation exposure (Close and Beadle, 2003). Exposure to UV- radiation promotes the production of foliar anthocyanins (Lindoo and Caldwell, 1978), and it has been hypothesized that anthocyanins provide a UV sunscreen (Lee and Lowery, 1980). UV responsive anthocyanins production in a rice cultivar was associated with a specific phase of phenylalanine ammonia lyase (PAL) biosynthesis (Reddy *et al.*, 1994). They focused that the anthocyanins induction in rice seedlings is mediated exclusively by the UV- component of sunlight.

Thus, the aim of this work was to investigate further growth changes and metabolic responses of garlic plants treated with UV_A, UV_C, as physical mutagens and sodium dodecyl sulphate (SDS), as chemical mutagens, throughout the entire period of the experiment.

MATERIALS AND METHODS

Time course experiment:

Homogenous bulblets of garlic (*Allium sativum* var. Seds 40) were used. The procedures of sterilization of bulblets, germination and growth of plants as well as the experiment set-up were the same as previously described by Shams-Eldeen (2008). After 14 days from the start of germination, the young vegetative plants were sub-divided into 6 subgroups, each of 5 pots, one of them was taken as initial, and the other 5 subgroups; one of them was left without treatment to serve as control and the other four subgroups, 2 were treated with 0.1M SDS and 0.3M SDS and the other 2 were exposed to UV- radiation day after day, for 2 hrs, with UV_A (365 nm) and UV_C (254 nm) throughout the duration of the experiment (Younis *et al.*, 2008).

Samples for determination of growth parameters, photosynthetic pigments (chl a, chl b, and carotenoids), UV-absorbing compounds (total

phenolic compounds and anthocyanins contents), total protein content and nucleic acid content (DNA and RNA), were taken at vegetative and flowering stages after 35 days and 60 days from transplantation. Leaf area was measured by square-paper method (Hasaneen *et al.*, 1994), fresh and dry masses measured after drying samples in an oven at 80°C to constant mass.

Plant photosynthetic pigments (Chl a, Chl b, and carotenoids) were determined in leaves of the test plants at initial, vegetative and flowering growth stages, by the method of Metzner *et al.* (1965).

Determination of UV-absorbing compounds (total phenolic compounds and anthocyanins): The total phenolic compounds were extracted and analyzed using the method of Malik and Singh (1980). Anthocyanins were extracted from oven-dried, ground garlic tissue samples of plants, suspended in 10 cm³ of acidified methanol and autoextracted at 0°C for 72 hrs in the dark with continuous shaking. Extracts were centrifuged for 10 min at 50.000 g then the absorbance was measured at 530 and 657 nm for each supernatant using Spekol spectro-colorimeter (Mirecki and Teramara, 1984).

Determination of nucleic acids: DNA was determined colorimetrically by the method of Sadasivam and Manickam (1996). RNA content was determined colorimetrically by the method of Devi (2000).

The results were statistically analyzed using the least significant difference (L.S.D.) at 5% level (Snedecor and Cochrain, 1980).

RESULTS AND DISCUSSION

Changes in growth parameters:

Treatment of garlic plants with 0.1M and 0.3M SDS induced significant variable decrease in shoot height, leaf area, fresh and dry masses through out the entire period of the experiment. On the other hand, root length of such treated plants showed significant increase. Exposure of garlic plants, at vegetative and flowering growth stages, to UV_A and UV_C radiation induces variable significant decrease in shoot length, leaf area, fresh and dry mass, whereas a significant increase in root length was apparent as given in (table 1).

In support of our results, Hamed (1990) and Hasaneen *et al.*, (1994) stated that under hydroponic culture conditions, the response of faba bean, castor bean, and rice plants to mutagens varied with the conditions and with the time of exposure to the chemical mutagenic substance. Stimulation of root elongation occurred in such treated plants after three weeks from treatment date.

Different species have different responses to the level of UV radiation (Skorska, 1996). The negative effects of UV_A and UV_C radiation result in different morphological parameters. Exposure to UV_A and UV_C decreased length of plumule and dry matter accumulation (Zuk-Golaszewska *et al.*, 2003). Dai *et al.* (1995) reported that after a few weeks of UV_B exposure, leaf area and plant dry weight of rice were significantly reduced.

UV_c	820.10*	630.70*	1450.80*	1.30*	539.40*	1910.20*
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As consequence of SDS and UV-treatment, in the literature, inhibition of hormone regulation (Bronoman and Teramura, 1993), protein synthesis (Kalbin *et al.*, 1997) and pigmentation (Musil, 1996) has often been observed. These responses are presumably due to a direct or indirect effect of SDS and UV-radiation on the activity of some enzyme systems. Furthermore, plant growth is primarily related to cell division and cell enlargement, and both processes are known to be controlled by plant growth regulators. Thus, the inhibition in growth of garlic plants as a result of SDS and UV-radiation appears to be correlated with hormonal biosynthesis in the affected plants.

Changes of photosynthetic pigments:

Low and high concentrations of SDS induced significant changes in Chl a, Chl b, Chl a+b, carotenoids and total pigment contents of garlic plants at vegetative and flowering growth stages (table 2). UV-radiation (A and C) of garlic plants showed significant decrease in Chl a, Chl b, Chl a+b, carotenoids and total pigment contents throughout the entire period of the experiment. Chl a/b ratio showed variable comparable values in control as well as in the treated plants throughout the entire period of the experiment (table 2).

Table 2: the effect of UV-radiation and sodium dodecyl sulphate (SDS) mutagens on metabolic changes; total pigments (mg100g⁻¹ fresh weight) of *Allium sativum* vegetative and flowering growth stages. Mean values are significantly different from control at P ≤ 0.05.

Parameter \ Treatment	Chl a	Chl b	Chl a+b	Chl a/b	Cars	Total pigments
Control	890.20	790.10	1680.30	1.13	415.70	2096.0
UV_c	810.60*	715.60*	1526.20*	1.13*	464.00*	1990.2*
0.1 M SDS	710.20*	580.70*	1290.90*	1.22*	409.20*	1680.1*
0.3 M SDS	710.20*	580.70*	1290.90*	1.22*	409.20*	1680.1*
Flowering stage						
Control	865.10	745.10	1610.20	1.16	420.40	2030.60
UV_A	790.10*	570.90*	1361.00*	1.38*	569.10*	1870.10*
0.1 M SDS	791.10*	586.00*	1377.10*	1.35*	548.60*	1796.00*
0.3 M SDS	665.30*	435.80*	1101.10*	1.53*	556.60*	1693.00*

In this connection, Yao and Liv (2007) demonstrated that enhanced UV-radiation significantly decreased chl a, chl b, chl a+b and carotenoids contents of *Picea asperata* plants. A parallel changing trend in Chl a and Chl b resulted in no significant changes in Chl a/b ratio under enhanced UV-radiation. Furthermore, the decreased tendency of Chl content and chl fluorescence appeared parallel to the biomass reduction in plants. The decrease in chl a+b content was mainly attributed to the destruction of chl b, which is more sensitive to radiation than chl a (Yao and Liv, 2007).

Similar results were also reported in previous publications (Casati *et al.*, 2002; Correia *et al.*, 2005). The decrease of total chl content in the present study may be due to the decreases of CO₂, since CO₂ protects chl from photooxidative destruction (Sing, 1946).

Carotenoids exert their protective functions as antioxidants to inactivate UV-induced radicals in the photosynthetic membranes (Gotz *et al.*, 1999). In the present study, the decrease in content of carotenoids suggested that involved UV-radiation or SDS-treatment caused considerable oxidative stress (table 2) by the accumulation of reactive oxygen species (ROS) (Yao and Live, 2007).

Changes in total phenolic compounds and anthocyanins content:

In garlic plants treated with low and high concentrations of SDS or exposed to UV_A and UV_C radiation, at vegetative and flowering growth stages, total phenolic compounds and anthocyanins content were significantly increased in comparison with control (table 3).

Table 3: the effect of UV-radiation and sodium dodecyl sulphate (SDS) mutagens on metabolic changes; total phenolic compounds (mg phenol eqv.100g dry wt) and Anthocyanins (mg anthocyanin100g⁻¹dry weight) of *Allium sativum* at vegetative and flowering growth stages. Mean values are significantly different from control at P ≤ 0.05.

Parameter Treatment	Total phenolic compounds	% of change	Anthocyanins	% of change
Control	130.6	0	86.1	0
UV _C	172.1*	31.78	93.1*	8.13
Control _{fl}	127.10	0	99.2	0
UV _C _{fl}	188.00*	47.95	110.10*	10.99
UV _A _{fl}	190.20*	49.65	128.70*	29.74

0.1 M SDS	188.40*	48.23	122.10*	23.08
0.3 M SDS	194.60*	53.11	136.20*	37.30

In accordance with the present results, UV-radiation and chemical mutagenic substances induce accumulation of a range of secondary metabolites, which in turn affect numerous physiological functions. Low florescence of UV stimulates the general phenylpropanoid pathway, resulting in accumulation of flavonoids and snapic esters (Day and Vogelmann, 1995).

Regulation of the biosynthesis of UV-screening flavonoids, total phenolic compounds and anthocyanins are at the level of transcription and is under the control of UV-photoreceptors (Greenloerg *et al.*, 1997). Depending on the species and developmental stage, red blue or UV wavelengths may play a role in anthocyanins synthesis through mediation of phytochrome or putative UV- receptors (Beckwith *et al.*, 2002). The UV inducibility of total phenolic compounds and anthocyanins and the ability of phenolic compounds to absorb UV-radiation have led investigators to postulate a UV protective role for UV-absorbing compounds, but there still are many questions that used to be assumed before a general UV-protective function can be ascribed to these compounds (Krizek, 2004).

Changes in nucleic acids content (DNA and RNA):

The nucleotide levels detected appeared to undergo same increase with an increase in the duration of growth period. As compared with control RNA and DNA contents, the UV- and SDS-treated plants showed a significant decrease throughout the entire period of the experiment (table 4). Thus in general, following sequence of treatments (0.3M SDS> 0.1M SDS> UV_A> UV_C) was displayed with respect to decrease in RNA and DNA contents of garlic plants, throughout the vegetative and flowering growth stages calculated as percentage of control (table 4).

Table 4: the effect of UV-radiation and sodium dodecyl sulphate (SDS) mutagens on metabolic changes; DNA (mg.100g⁻¹fresh mass) and RNA (mg.100g⁻¹dry weight) of *Allium sativum* at vegetative and flowering growth stages. Mean values are significantly different from control at P ≤ 0.05.

parameter Treatment	% of change		% of change	
	DNA		RNA	
Control	109	0	134	0
UV_C	104*	-4.59	131*	-2.24
UV_A	94*	-13.76	124*	-7.46
0.1 M SDS	101*	-7.34	129*	-3.73
0.3 M SDS	91*	-16.51	120*	-10.45
Flowering stage				
Control	127	0	149	0
UV_C	121*	-4.72	144*	-3.36
UV_A	112*	-11.81	128*	-14.09
0.1 M SDS	119*	-6.30	145*	-2.68
0.3 M SDS	108*	-14.96	126*	-15.43

In support of the present results, Hidema and Kumagai (2006) and Saleh *et al.*, (2006) detected a significant change in both RNA and DNA of *Oryza sativa* seedlings and soybean cultivars exposed to UV_A and UV_B radiation. Furthermore, in certain tissues, UV-radiation has been shown to interfere with processes such as transcription and replication, resulting in reduction of RNA synthesis (Sancar *et al.*, 2004; Hidema and Kumagai, 2006).

The present results (table 4) can be explained simply on the basis that the most common inactivation of nucleic acids by UV-radiation is through photochemical lesions involving polymers of pyrimidine bases in the deoxyribonucleic acids (DNA). The result is the production of pyrimidine dimers and loss of DNA biological activity. It is evident that UV-radiation can cause acceleration in the mutation rates and aberrations of chromosomes (Kalbin *et al.*, 1997). Repair systems for the DNA molecules have been found in plants, however, and involve the enzymatic splitting of the dimers formed by UV-absorption and SDS-treatment. Such a repair system has been implicated in repairing epidermal tissue damage, restoration of growth rate and biosynthesis of chlorophyll (Lainsen and Micheal, 1998).

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**التأثيرات الفسيولوجية و الكيموحيوية للأشعة فوق البنفسجية و دودوسايل سلفات
الصوديوم (SDS) كمطفرات على نمو و أبيض نبات الثوم
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قسم النباتة و صنملا ءعماد - مولعللا ءمبلك -**

تم فى هذا البحث دراسة تأثير كلا من الأشعة فوق البنفسجية و ال SDS كمطفرات فيزيائية و كيميائية على نمو و أبيض نبات الثوم خلال مرحلتى النمو الخضرى و الازهار. و لقد أظهرت نتائج التجارب أن تعريض النباتات للأشعة فوق البنفسجية من النوع أ؛ ج وكذلك معاملة النباتات بتركيز ٠.١ جزئ و ٠.٣ جزئ من مادة ال SDS خلال مراحل النمو الخضرى و الازهار، أدى الى حدوث نقص معنوى فى دلالات النمو المختلفة -- طول المجموع الخضرى- مساحة سطح الورقة- الوزن الطازج و الوزن الجاف بينما لوحظ زيادة معنوية فى طول الجذر للنباتات المعاملة بالمطفرات الكيميائية (SDS) و المطفرات الفيزيائية (UV-radiation) أثناء مرحلتى النمو الخضرى و الازهار. كذلك لوحظ وجود نقص معنوى فى محتوى النباتات من كلورفيل أ، كلوروفيل ب، كاروتينات، كلورفيل أ+ب و محتوى الأصباغ الكلية المعاملة بكل من ال SDS بتركيزه و كذلك المعرضه للأشعة فوق البنفسجية من النوع أ، ج خلال مرحلتى النمو الخضرى و الازهار. بالاضافة الى ذلك وجد أن معاملة النباتات بالمواد المطفرة سواء الكيميائية أو اى فيزيائية أدى الى زيادات معنوية فى محتوى النباتات من المواد الفنتولي% الكلية و محتوى صبغ الانثوسيانين أثناء مرحلتى النمو و الازهار اذا ما قورنت بمثيلاتها الغير معاملة، كذلك وجد أن محتوى النباتات من الأحماض اى نووية (DNA, RNA) ينقص تدريجيا بمعاملة النباتات بالمطفرات الكيميائية (SDS) و كذلك تعريض النباتات للمطفرات الفيزيائية UV (radiation) أثناء مرحلتى النمو الخضرى و الازهار اذا ما قورنت بمثيلاتها الغير معاملة. و لقد تم مناقشة النتائج المتحصل عليها فى ضوء الميكانيكات المتنلفة لتأثير المطفرات الكيميائية (SDS) و المطفرات الفيزيائية UV (radiation) على ابيض الثوم و أثر ذلك فى النمو و لأبيض للنبات.

Table 1: The effect of UV-radiation and sodium dodecyl sulphate (SDS) mutagens on growth parameters ; length of root (cm plant⁻¹) length of shoot (cm plant⁻¹), leaf area (cm² plant⁻¹), fresh mass (g plant⁻¹), dry mass (g plant⁻¹) and relative growth index (%) of *Allium sativum*, at vegetative and flowering growth stages.

Mean values are significantly different from control at P < 0.05.

Parameter Treatment	Length of shoot % change	Length of root % change	% change	Leaf area	% change	Fresh mass	% change	Dry mass	% change	RGI	RGI
Initial	1.6	0	6.26	0	54)	0	2.94	0	0.6	0	0
Control	4.02	0	7.78	0	22.45	0	5.46	0	0.71	0	0
UV _C	2.55*	-36.57	9*60*	23.39	21.02*	-6.37	4.17*	-23.63	0.57*	-27.85	72.15
UV _A	2.62*	-34.83	10.90*	40.10	1(.62*	-17.06	4&91*	-10&07	0.70*	-1.39	88.61
0.1 M SDS	2.65*	-34.08	8.70*	11.83	20.47*	-8.82	4.55*	-16.47	0.63*	-20.25	79.75
0.3 M DS	2.37*	-41.05	6.60*	23.39	19.07*	-15.06	4.60*	-15.75	0.66*	-16.46	83.14
Flowering stlce											
Control	5.36	0	8.40	0	27.50	0	16.32	0	5.84	0	0
UV _C	3.43*	-36.01	10&10*	20.24	25&74*	-6.40	14.82*	-9.19	4.48*	23*29	
UV _A	3.08*	-33.21	11.40*	35.71 24.96 -3.03 24.96 -3.03	14.69*	-9.99	4.77*	-18.32	81.68		
0&1 M SDS	3.04*	-43.0	9.80*	16.67	20.90*	-5.82	13.6*	-17.52	4.55*	-22.09	77.91
0.3 M SDS	3.10*	-42.16	10.60*	26.19	24.24*	-5.83	14.00*	-14.22	4.71*	-19.35	80.65

Relative growth index = (dry wt of treated sample / dry wt of controlled sample) x 100