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Effect of Heat Stress on Fatty Acids Profiles of *Aloe vera* and *Bryophyllum pinnatum* Leaves

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Abstract: Plants are exposed to various environmental stresses which lead to a number of physiological and biochemical changes. One of the most usual forms of environmental stress encountered by plants is heat stress. The present study was therefore conducted to investigate the effects of heat stress on the fatty acids profiles of *Aloe vera* and *Bryophyllum pinnatum* leaves. Fourteen plants of each species were investigated in the present study. The plants were then divided into two groups, each group comprising of seven plants. One group from each species was kept under control environmental conditions with daily temperature of 28 ± 2°C while the other set was kept in green house where average daily temperature ranged between 38°C and 43°C. After 15 days leaves were collected from both groups of plants. Lipids were extracted from the leaves. The fatty acids were analyzed by Gas Chromatography Mass spectrometry. Results exhibited that Palmitic acid was found to be the most abundant fatty acid in the leaves of both set of plants; the second most abundant fatty acids were linoleic acid and linolenic acid, while lauric acid was present in the lowest concentrations. Significant changes were observed in the concentration of fatty acids in response to heat stress. The concentration of saturated fatty acids like palmitic acid and stearic acid increased while unsaturated fatty acids like linoleic acid and linolenic acid become decreased in response to heat stress. In both plant species total saturated fatty acids increased while total unsaturated fatty acids became decreased significantly with heat stress.

Key words: Heat stress · Fatty acids · Plant leaves

INTRODUCTION

Plants face several environmental stresses and must have the ability to adapt accordingly because they cannot escape from the environmental stresses through locomotion. Plants adapt to these stresses through various approaches such as phenology, morphology and modification in physiological and biochemical pathways [1]. High temperature is one of the most usual forms of environmental stress encountered by plants and heat stress caused by high temperatures can have harmful effects on them [2].

Heat or temperature stress affects the fatty acid composition in leaves [3]. Plant cell plasma membrane is a direct and most important target of heat stress. Plant cells can modify the saturation level of lipids in the membrane to increase membrane thermo-stability and consequently increase the ability of the whole plant to

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tolerate heat stress [4, 5]. Heat stress produces various changes in living cells that eventually affect membrane structure and functions [6].

Aloe vera and *Bryophyllum pinnatum* are two succulent, perennial herbs; *Aloe vera* leaves are thick and contain water. The leaves of *Bryophyllum pinnatum* are freshly dark green. These plants can survive for a long periods of drought [7, 8] therefore understanding the mechanism they use in order to counterbalance heat stress may be helpful in understanding the mechanism of plants use to adapt heat stress. The present study was aimed to determine the effect of heat stress on fatty acids profiles of *Aloe vera* and *Bryophyllum pinnatum* leaves.

MATERIALS AND METHODS

The experiment was carried out at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture, KPK Peshawar and Pakistan Council of Scientific and Industrial Research (PCSIR) Peshawar. Specimens of *Aloe vera* and *Bryophyllum pinnatum* were collected from Elite garden Hayatabad Peshawar and kept in the green houses at IBGE, The University of Agriculture Peshawar. A total of 28 plants were used in the study 14 from *Aloe vera* and 14 from *Bryophyllum pinnatum*. The plants were divided into two groups (A and B). Group A consisted of fourteen plants, seven of each species. These plants were kept in the growth room under control temperature maintained at 28 ± 2°C. Group B was also consisted of 14 plants, seven of each species *viz., Aloe vera* and *Bryophyllum pinnatum*. These plants were then kept in the green house. The temperature was recorded with the help of thermometer, which ranged between 38 and 43°C. The plants were well watered. Plants leaves were used as a sample for extraction and analysis of fatty acids. After 15 days leaves from both groups of plants were collected from three plants. Each sample was collected from three different leaves of a single plant. Lipids were extracted in a mixture of chloroform and methanol [9]. The lipids were converted to fatty acids methyl esters (FAMEs) [10]. Fames were analyzed with the help of Gas chromatography-mass spectrometry (GC-MS) Shimadzu-QP 2010 Japan, set with electron impact detector. The concentration of each fatty acid was calculated as a percent of the total fatty acids present in the sample.

Statistical Analysis: The data obtained was analyzed using SPSS to the general linear model (GLM) procedure of statistical analysis system. The means were compared by LSD and all pair wise comparison test. Significance was found at ($P<0.05$).

RESULTS AND DISCUSSION

A total of seven fatty acids (*viz.,* lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid) were identified in the leaves of both plant species. Out of these four were saturated fatty acids and three were unsaturated fatty acids. Highest concentration was recorded for palmitic acid followed by linolenic acid while lowest concentration was observed for lauric acid. Significant changes in the fatty acid concentrations were observed between normal and heat-stressed plants. In agreement with our results higher concentration of palmitic acid was recorded in pumpkin, watermelon and some other plant species [11]. Similar results have been reported in sixteen different species of carex [12] and in Bermuda grass [13]. Contrary to our findings higher concentrations for linolenic acid and linoleic acid were reported in tomato leaves [14]. The variation in the results may be due to different plants species and environment.

The effect of heat stress on the saturated fatty acids in *Aloe vera* whole leaf is presented in the Table 1. It is obvious from the results that the concentration of lauric acid slightly decreased while that of palmitic acid and stearic acid increased significantly ($p<0.05$) with heat stress. Overall significant increase (26.1%) was recorded in the concentration of total saturated fatty acids (TSFAs) in leaves of *Aloe vera* with heat stress. In *Bryophyllum pinnatum* the concentrations of palmitic acid and stearic acid were found to be increased significantly with heat stress while no significant ($P>0.05$) effect was visible on myristic acid (Table 1). Overall the total saturated fatty acid concentration in *Bryophyllum pinnatum* leaves of normal plants were found significantly less than that of heat-stressed plants. The concentration of lauric acid decreased in response to heat stress. In agreement with our results increase in the concentrations of palmitic acid was also found in arabidopsis plants exposed to elevated temperatures [15]. Similar increase in the concentration of saturated fatty acids [16] at high growth temperatures have also been noticed in *Atripex lentiformis*. The increase in the concentration of saturated fatty acids is an adaptive response in plants against heat stress [17].

Among the unsaturated fatty acids in *Aloe vera* leaf highest concentration (18.78%) was recorded for linolenic acid followed by linoleic acid (11.89%) and the lowest values (7.26%) were recorded for oleic acid.

Table 1: Concentration (Means ± SE) of saturated fatty acids in leaves of normal and heat-stressed *Aloe vera* plants.

Fatty acid	Normal	Stress	P-Value
Lauric acid	$1.71^{a} \pm 0.120$	$0.86^{b} \pm 0.038$	0.002
Myristic acid	$3.23^{a} \pm 0.759$	$3.59^{a} \pm 0.441$	0.701
Palmitic acid	$40.84^{b} \pm 0.840$	$51.82^{a} \pm 0.213$	0.000
Stearic acid	$15.44^{b} \pm 0.755$	$20.94^{a} \pm 0.801$	0.007
TSFAs	$61.24^{b} \pm 0.983$	$77.23^{a} \pm 0.934$	0.000

The data with different superscripts in the row represents significant (P<0.05) effect.

TSFAs: Total saturated fatty acids.

Table 2: Concentration (Mean ± SE) of the saturated fatty acid in*Bryophyllumpinnatum* leaves of normal and heat-stressed plants.

Fatty acids	Normal	Stress	P- value
Lauric acid	$0.97^{a} \pm 0.112$	$0.52^{b} \pm 0.033$	0.021
Myristic acid	$4.56^{a} \pm 0.719$	$4.62^{a} \pm 0.890$	0.959
Palmitic acid	$46.9^{b} \pm 1.362$	$58.30^{a} \pm 1.038$	0.001
Stearic acid	$12.92^{b} \pm 0.567$	$16.55^{a} \pm 1.293$	0.035
TSFAs	$65.46^{b} \pm 1.366$	$80.01^{a} \pm 0.607$	0.000

Means with different superscripts within the row are significantly different at p<0.05.

TSFAs: Total saturated fatty acids

Table 3: Concentration (Means ± SE) of unsaturated fatty acids in *Aloe vera*leaves of normal and heat-stressed plants.

Fatty acid	Normal (Concentration %)	Stress (Concentration %)	P-Value
Oleic acid	$7.26^{a} \pm 0.442$	$7.00^{a} \pm 0.537$	0.727
Linoleic acid	$11.89^{a} \pm 0.781$	$7.26^{b} \pm 0.442$	0.008
Linolenic acid	$18.78^{a} \pm 0.766$	$8.75^{b} \pm 0.730$	0.000
TUSFAs	$37.94^{a} \pm 0.570$	$22.77^{b} \pm 0.934$	0.000

The data with different superscripts represents significant (P<0.05) effect.

TUSFAs: Total unsaturated fatty acids.

Table 4: Concentration (Mean ± SE) of unsaturated fatty acid in *Bryophyllumpinnatum* leaves of normal and heat-stressed plants.

Fatty acids	Normal(Concentration %)	Stress (Concentration %)	P- value
Oleic acid	$7.92^{a} \pm 0.626$	$3.83^{b} \pm 0.211$	0.003
Linoleic acid	$10.53^{a} \pm 0.442$	$9.80^{a} \pm 1.048$	0.506
Linolenic acid	$13.90^{a} \pm 0.894$	$6.76^{b} \pm 0.767$	0.002
TUSFAs	$32.35^{a} \pm 1.412$	$20.41^{b} \pm 0.711$	0.001

Means with different superscripts within the row are significantly different at p<0.05.

TUSFAs: Total unsaturated fatty acids.

The data presented in Table 2 showed that all unsaturated fatty acids decreased in heat-stressed plants. The concentrations of linoleic and linolenic acid were significantly decreased in plants exposed to heat stress. The concentration of total unsaturated fatty acids decreased from 37.94 to 22.77% in plants treated with heat stress. The data pertaining to the effect of heat stress on unsaturated fatty acids in *Bryophyllum pinnatum* leaves is shown in the Table 3. The concentration of linolenic acid and oleic acid were significantly decreased in heat-stressed plants whereas no significant decrease was observed in the concentration of linoleic acid. The data showed significant decrease in the concentration of unsaturated fatty acids in plants exposed to heat stress (Table 2). Similar to our results a decrease of 10-20% in the concentration of linolenic acid was observed in *Atripex lentiformis* with temperature stress [16]. Similarly, a reduction of 57% in the concentration of linoleic acid and 13% decrease in double bond Index [18] were found in the leaves of creeping bent grass (*Agrostis palustris*) exposed to high soil temperatures. The decrease in concentration of unsaturated fatty acids may be due to the fact that fatty acids with more double bonds are easily attacked by free radicals [19].

Overall in both plant species increase in saturation of fatty acids was observed at high temperatures. The increase in saturated fatty acids of membranes has been associated with increase in melting temperature which provides heat tolerance. An arabidopsis mutant accumulated large amounts of palmitic acid in chloroplast lipids which increased the optimum growth temperature [20]. The saturation of membrane lipid is considered as significant factor in conferring tolerance to high temperature [21]. The modifications in the composition of membrane lipid may also be accredited to stress-induced degradation processes. Enzymes such asphospholipase and lipoxygenase may be involved in these degradation processes [22].

CONCLUSIONS

A higher concentration of saturated fatty acids in *Aloe vera* and *Bryophyllum pinnatum* leaves seem to perform a significant role in heat-tolerance of these plants. It was observed in the study that at high temperatures plants tend to produce more saturated fatty acids and decrease the production of unsaturated fatty acids. A detailed study is needed to evaluate the mechanism of shift in plants machinery from production of saturated to unsaturated fatty acids. Understanding the mechanisms of heat tolerance in plants and triggering similar mechanism in other plants can provide help in reducing the harmful effect of global warming on crop plants.

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