International Journal of Biological Technology ISSN: 0976 - 4313 (Print)

Effect of heat stress on free amino acids in leaves of *Aloe vera* and *Bryophyllum pinnatum*

BABITA Kumari[1] and PRASHANT Kumar Roy[2]

[1]Faculty of Sciences, Indira Gandhi Technological and Medical Sciences University, Ziro, Arunachal Pradesh (India)
[2]Department of Paramedical, Indira Gandhi Technological and Medical Sciences University, Ziro, Arunachal Pradesh (India)
Email: bbtmshr@yahoo.co.in
Received: 11 March, 2019 / Accepted: 30 March, 2019/ Published Online:15 April, 2019

http://www.gtrpcompany.com/ijbt.htm

Citation: Babita Kumari and Prashant Kumar Roy. Effect of heat stress on free amino acids in leaves of *Aloe vera* and *Bryophyllum pinnatum*. Inter J Biol Technology, 2019; 10(1):1-5.

Abstract

In the present study the effects of heat stress on the free amino acid profiles of *Aloe vera* and *Bryophyllum pinnatum* leaves were investigated. Heat stress was provided by exposing the plants to a daily temperature of 38-43 ^{0}C for one month. After heat stress the leaves were collected and dried in oven. The powdered dried leaves were used for amino acids extraction and analysis. In *Aloe vera* leaves 9 essential and 7 non-essential amino acids were detected, highest concentration was recorded for phenylalanine followed by arginine and valine while histidine and aspartate were present in the lowest amounts. In *Bryophyllum pinnatum* 8 essential and 7 non-essential amino acids were detected, glycine was recorded in highest concentrations followed by arginine and proline while lysine was observed in the lowest concentrations. The two plants accumulated different amino acids in response to heat stress. In both plant species proline and serine were accumulated while the concentration of alanine and glycine declined significantly in response to heat stress. The total essential amino acids decreased while total non-essential amino acid increased significantly in *Aloe vera* while in *Bryophyllum pinnatum* no significant effect was observed on the concentration of total essential and total non-essential amino acids in response to heat stress.

Keywords: Plants leaves, Heat stress, Free amino acids

1 INTRODUCTION

Plants are exposed to a variety of environmental stresses during their life. As plants lack the ability of locomotion, they adapt themselves to their environment through various approaches such as changes in phenology, morphology and alterations in physiological and biochemical states [1]. Temperature is the most inconsistent stress encountered by plants and heat stress caused by high temperatures can have a detrimental effect on them [2]. Environmental temperatures also influence the distribution of the plant [3]. Abiotic stress leads to a chain of physiological, morphological, molecular and biochemical changes that adversely affect the development and yield of the plant [4]. Plants adjust to stress conditions by regulating definite sets of genes in response to stress signals, which differ depending on factors such as the harshness of stress, other environmental factors, and the plant type [5]. Plants accumulate remarkably soluble organic compounds of low molecular weight, known as compatible solutes, in response to salt, drought and heat stress. These organic compounds are present in stable form within the plant cells and cannot be metabolized with ease [6]. The amplification of these compounds may possibly help in increasing internal osmotic pressure and reducing water loss from the cell. Common compatible solutes contain mannitol, other sugar alcohols, amino acids such as proline, and amino acids derivatives. A few of these compatible solutes, such as proline, are accumulated in approximately all plant species [7]. Increase in concentration of amino acids has been determined in several studies on plants subjected to abiotic stresses [8, 9].

Aloe vera is a succulent perennial herb, belongs to the Liliaceae family, having a whorl of elongated pointed leaves. *Aloe vera* leaves are thick and contain the water supply [10]. The thick leaves of *A. vera* assists the plant to survive long periods of drought. This plant can stay alive in very warm dry climates, where most other vegetation fades away, due to the high-water preserving capacity of its leaves. *A. vera* contains amino acids, lipids, tannins, sterols, sugars and flavonoids. *Bryophyllum pinnatum* is an erect, succulent and perennial plant. It belongs to the class rosopsida and family Crassulaceae that grows about 1.5 meter high above the ground. It reproduces through seeds as well as vegetatively from leaf bulbils. The leaves of *Bryophyllum pinnatum* are freshly dark green. These plants were investigated in the present study for effect of heat stress because they possess the mechanisms to survive long periods of drought [11].

2 MATERIALS AND METHODS

A total of 32 plants were used in the study 16 from *Aloe vera* and 16 from *Bryophyllum pinnatum*. The normal plants were kept in the growth room under control temperature maintained at 28 ± 2 ^{0}C while for providing heat stress the plants were grown in green house at a temperature of 38-43 ^{0}C. Leaves were collected from both normal and heat-stressed plants. The leaves were washed with water of the tap to take away any impurities and then kept in oven at 40 ^{0}C for drying. The dried leaves were ground with the help of pestle and mortar. Extraction of amino acids was carried out in 0.1N HCl according to the method of Mansfield and Baerlocher [12]. The extract was kept in refrigerator at -20^{o}C for storage purpose. Each sample was taken in triplicates. The extract was diluted by adding 0.1N HCl and filtered through membrane. Ten micro liters from the sample was taken with the help of calibrated syringe and injected into the Amino acid analyzer (Shimadzu LC-10 Avp) inlet for analysis.

2.1 Instrument configuration and Analytical conditions for amino acids analysis

Degasser: DGU-14A, Pump: LC-10Atvp, Injector: SIL-10Advp, column Oven: CTO-10Acvp, Detector: RF-10Avp, Software: LC solution Ver. 1.21 SP1. Flow rate: 0.4ml/min, Column temperature: 60 ^{0}C, Detection: Fluorescence, injection volume: 10µl. The following mobile phases were used;

Mobile phase-A (Sodium citrate 0.2 N, pH-3.20), Mobile phase-B (Sodium citrate 0.6 N, pH = 10.0 and 0.2 M H_3BO_3) and Mobile phase-C (Sodium hydroxide, 0.2 M)

2.2 Buffer solution

Na_2CO_3 (340.7 g) was weighed and 600ml of Double distilled water was added to it. Now added 13.57 g of H_3BO_3 to it and shaken vigorously in order to dissolve all the crystals.

2.3 Reaction Solution-A

Took 500 ml from the above buffer solution, and 0.2 ml of 7-10% sodium hypochlorite (NaClO) was added to it, stirred and filtered through 0.45 µm membrane filter.

2.4 Reaction Solution-B

OPA (O-Phthaldehyde) at the rate of 0.4 g was dissolved in 7 ml of ethanol. A volume of 450 ml of the buffer solution was taken and ethanol was added to it. Now 0.5 g of N-acetyl cysteine was weighted and added to the buffer solution. The solution was shaken well till all the crystals got dissolved, and then filtered through 0.45 µm membrane filter.

2.5 Statistical Analysis

The data 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).

3 RESULTS AND DISCUSSIONS

3.1 Essential amino acids in *Aloe vera* leaves

Among the essential amino acids highest concentrations were recorded for arginine (10.9%) followed by valine (10.7%) while lowest concentrations were recorded for histidine (0.95%). The concentration of histidine and threonine significantly increased with heat stress while those of arginine, leucine, phenylalanine and valine decreased significantly. The effect of heat stress on essential amino acids in *Aloe vera* leaves is demonstrated in the (Table 1). Total essential amino acids decreased significantly with heat stress. In agreement with our results Hassanein et al. [13] also observed decrease in the concentration of essential amino acids in *Triticum aestivum*.

Table -1: Concentrations (Means ± SE) of essential amino acids in leaves of control and heat-stressed *Aloe vera* plants

Amino acid	Normal plants (% Conc.)	Stressed plants (% Conc.)	P-value
Arginine	20.9^a ± 0.432	6.42^b ± 0.419	0.005
Histidine	0.85^b ± 0.252	1.98^a ± 0.112	0.006
Isoleucine	6.12^a ± 0.798	7.10^a ± 1.000	0.167
Leucine	3.55^a ± 0.758	3.93^a ± 0.426	0.798
Methionine	6.20^a ± 1.019	7.22^a ± 1.013	0.670
Phenylalanine	13.2^a ± 0.700	4.230^b ± 0.300	0.000
Threonine	1.25 ± 0.119	4.790^a ± 0.342	0.000
Valine	10.6^a ± 0.351	7.27^b ± 0.431	0.001
Lysine	3.58^a ± 0.687	2.99^a ± 0.382	0.7.02
TE Amino acids	49.6^a ± 1.182	52.36^b ± 1.170	0.010

Means with different superscripts within the row are significantly different at p<0.05. TEAs: Total Essential amino acids.

Essential amino acids in *Bryophyllum pinnatum* leaves

In *Bryophyllum pinnatum* leaves arginine (13.9%) was the major amino acid while the other most abundant amino acids were Methionine (8.2%) and Isoleucine (7.4%). The effect of heat stress on essential amino acids in *Bryophyllum pinnatum* is presented in the (Table 2). It is clear from the table that among the essential amino acids in the leaves of *Aloe vera* the concentration of lysine and leucine increased significantly in plants exposed to heat stress while the concentration of histidine and isoleucine decreased significantly. No significant effect was observed on the concentration of arginine, valine, methionine and phenylalanine.

Table -2: Concentration (Means ± SE) of essential amino acid in *Bryophyllum pinnatum* leaves of normal and heat-stressed plants

Amino acids	Normal plants (% Conc.)	Stressed plants (% Conc.)	P-value
Arginine	12.99[a] ± 0.827	14.11[a] ± 0.724	0.71
Histidine	3.84[a] ± 0.355	1.82[b] ± 0.578	0.50
Isoleucine	6.59[a] ± 0.761	3.99[b] ± 0.729	0.05
Leucine	3.30[b] ± 0.347	5.44[a] ± 0.484	0.02
Methionine	8.25[a] ± 0.292	6.30[a] ± 0.731	0.08
Phenylalanine	0.97[a] ± 0.334	5.02[a] ± 0.817	0.29
Valine	6.97[a] ± 0.382	4.18[a] ± 0.607	0.19
Lysine	1.47[b] ± 0.141	5.96[a] ± 0.852	0.00
TE Amino Acids	59.48[a] ± 1.925	48.90[a] ± 1.2003	0.78

Means with different superscript show significant effect on the concentration of the amino acid. TEAs: Total Essential amino acids.

Table -3: Concentration (Means ± SE) of non-essential amino acids in leaves of normal and heat-stressed *Aloe vera* plants

Amino acid	Normal plants	Stressed plants	P-value
Alanine	5.45[a] ± 0.397	1.58[b] ± 0.150	0.000
Aspartate	1.05[b] ± 0.315	6.79[a] ± 0.504	0.000
Glutamate	4.20[b] ± 0.430	10.82[a] ± 0.793	0.001
Glycine	8.79[a] ± 0.421	3.00[b] ± 0.475	0.000
Proline	4.02[b] ± 0.658	9.37[a] ± 0.590	0.003
Serine	3.26[b] ± 0.509	7.88[a] ± 0.858	0.009
Tyrosine	4.59[a] ± 0.763	4.84[a] ± 0.789	0.531
TNE Amino acids	30.30[b] ± 1.020	3.37[a] ± 1.046	0.001

Means with different superscripts within the row represents significant effect ($p<0.05$).
TNEAs: Total non-essential amino acids.

Upon exposure to heat stress the concentrations of essential amino acids like histidine and threonine increased significantly in *Aloe vera* while leucine and lysine increased in *Bryophyllum pinnatum* leaves. Similar results have been reported by Mansour [14], and Allen et al. [15], who observed increase in concentration of leucine and other amino acids in plants exposed to drought and salt stress. On the other hand, higher concentration of arginine, phenylalanine and valine were observed in normal plants as compared to heat-stressed plants in *A. vera* while in *Bryophyllum pinnatum* decrease was observed in the concentrations of histidine and isoleucine. Our results are supported by the data in literatures willadino et al. [16].

3.3 Non-essential amino acids in *Aloe vera* leaves

Among the non-essential amino acids highest concentration was recorded for glycine (8.7%) followed by tyrosine (6.5%) while the lowest concentration was observed for aspartate (1.05%). The concentrations of alanine and glycine significantly decreased under heat stress while the concentration of aspartic acid, glutamic acid, proline, serine and threonine increased significantly under heat stress. The effect of heat stress on non-essential amino acids in *Aloe vera* leaves is displayed in the (Table- 3).

For non-essential amino acids in *Bryophyllum pinnatum* leaves highest concentration was recorded for glycine (15.0%) the second most abundant amino acids were proline (10.2%) and alanine (5.2%) while glutamate (2.3%) was present in the lowest concentrations (Table 4). Among the non-essential amino acids the concentration of proline and serine increased significantly in both plants while in *Aloe vera*, aspartate and glutamate also increased with heat stress. The increase in concentration of histidine, proline and glutamic acid are confirmatory to the findings of Hassanein et al. [13], who also observed similar results in *Triticum aestivum* in response to heat stress. Similarly, accumulation of proline and other nitrogen containing compounds have been reported by Rabe [17], and Kasim [18], in crops such as barley, oat and peas subjected to environmental stress.

Table -4: Concentration (Means ± SE) of non-essential amino acid in leaves of control and heat-stress *Bryophyllum pinnatum* plants

Amino acids	Normal plants (% Conc.)	Stressed plants (% Conc.)	P-value
Alanine	$4.32^a \pm 0.573$	$1.64^b \pm 0.383$	0.00
Aspartate	$4.52^a \pm 0.726$	$5.43^a \pm 0.904$	0.07
Glutamate	$2.06^a \pm 0.391$	$4.39^a \pm 1.070$	0.21
Glycine	$14.34^a \pm 0.700$	$3.74^b \pm 0.474$	0.00
Proline	$10.28^b \pm 0.212$	$19.37^a \pm 0.927$	0.00
Serine	$3.22^b \pm 0.854$	$6.05^a \pm 0.613$	0.03
Tyrosine	$4.80^a \pm 0.449$	$1.35^b \pm 0.413$	0.00
TNE Amino Acids	$44.56^a \pm 2.178$	$47.07^a \pm 3.094$	0.54

Means with different superscript show significant effect on the concentration of the amino acid. TNEAs: Total non-essential amino acids.

In both *Aloe vera* and *Bryophyllum pinnatum* the concentrations of proline and serine increased while that of alanine and glycine decreased significantly with heat stress. Plants respond to conditions of stress by increasing osmotic potential through increase in synthesis of compatible osmolytes within their cells [19]. Correspondingly, Paleg et al. [20], stated that serine and proline are important for increasing tolerance to drought, salt, and heat stress. Jager and Meyer [21], suggested that proline acts as a storage compound of carbon as well as nitrogen for quick recovery from stress. In agreement to our observations; changes in the concentration of free amino acids in different parts of the plants, in response to heat stress, have also been reported by [14, 22]. According to Yancy et al. [6], amino acids are one of the osmo-protectants so accumulation of amino acids in plants in response to heat stress might help the plant escape osmotic stress. Proline is the most important amino acid which accumulated in the present study in response to heat stress which can be associated with increase in thermo-tolerance [23].

3.4 Conclusions

The increase in concentration of proline and serine in *Bryophyllum pinnatum* and *Aloe vera* leaves in response to heat stress may be a significant feature of cellular protection against heat stress in these plants. Different species of plants tends to accumulate different amino acids in response to heat stress. More Studies are needed to know the temperature at which accumulation of amino acids starts in plant leaves and the levels of amino acids accumulated at different stages of growth.

5 REFERENCES

1. Allen GG, Steven T, Chinski TZ. The effects of drought stress on free amino acids accumulation and protein synthesis in *Brassica napus*. *Plant Physiol.* 2006; 90(1): 9-14.
2. Claussen W. Proline as a measure of stress in tomato plants. *Plant Sci*. 2005;168: 241-248.
3. Hassanein RA, El-Khawas SA, Ibrahim SK, El-Bassiounty HM, Mostafa HA, El-Monetm AA. Improving the thermo tolerance of wheat plant by foliar application of Arginine or putrescine. *Pak. J. Bot*. 2013;45 (1): 111-118.
4. Jager HJ, Meyer HR. Effect of water stress on growth and proline metabolism of *Phaseolus vulgaris* L. *Oecologia*. 1977;30: 83-96.
5. Kasim WA. Amino acid and protein profiles of *Vicia faba* salt-stressed seedlings grown from thermally-stressed seeds. *Ind. J. Plant Physiol*.2006;11: 364-372.
6. Koh I. Acclimative response to temperature stress in higher plants: Approaches of gene engineering for temperature tolerance. *Plant Biol*.2002; 53: 225-45.
7. Lugan R, Niogret MF, Leport L, Guegan JP, Larher FR, Savoure A, Kopka J, Bouchereau A. Metabolome and water homeostasis analysis of *Thellungiella salsuginea* suggests that dehydration tolerance is a key response to osmotic stress in this halophyte. *The Plant J*. 2010;64: 215-229.
8. Mansfield D, Baerlocher MO. Free amino acids. Canada research chair in wood and fibre quality, department of wood sciences, University of British Columbia. Springer Neatherlands. Chap. 2005;10: 69-74.
9. Mansour MMF. Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol. Plant*. 2000; 43: 491-500.
10. Okwu DE, Uchenna NF. Multifaceted medicinal plants of drug and pharmaceutical industries. *Afr. J. Biotechnol*. 2009;8 (25): 7271-7282.
11. Paleg LG, Douglas TJ, Daal AV, Keech DB. Proline, betaine and other organic solutes protect enzymes

against heat inactivation. *Aust. J. Plant Physiol*. 1981;8: 107-112.

12. Penfield S. Temperature perception and signal transduction in plants. *New Phytologist,* 2008;179: 615-628.
13. Rabe E. Stress physiology: the functional significance of the accumulation of nitrogen-containing compounds. *J. Horti. Sci*. 1990; 65(3): 231-243.
14. Robinson SP, Jones GP. Accumulation of glycine betaine in chloroplasts provides osmotic adjustment during salt stress. *Aust. J. Plant Physiol*. 1986;13: 659-68.
15. Sarkadi SL, Kocsy G, Varhegyi A, Galiba G, Ronde JAD. Stress induced changes in the free amino acid composition in transgenic soybean plants having increased proline content. *Biol. Plant*. 2004; 50(4): 793-796.
16. Shevyakova NI, Roshchupkin BV, Paramonova NV, Kuznetsov VV. Stress responses in *Nicotiana sylvestris* cells to salinity and high temperature. 1. Accumulation of proline, polyamine, betaines and sugars. *Russ. J. Plant Physiol.* 1994; 41: 490-496.
17. Strickland FM, Kuchel JM, Halliday GM. Natural products as aids for protecting the skin's immune system against UV damage. *Cutis.* 2004;74 (5): 24-28.
18. Sung DY, Kaplan F, Lee KJ, Guy CL. Acquired tolerance to temperature extremes. *Trends in Plant Sci*. 2003; 8: 179-187.
19. Usadel B, Blasing OE, Gibon Y, Poree F, Hohne M, Gunter M, Trethewey R, Kamlage B, Poorter H, Stitt M. 2008. Multilevel genomic analysis of the response of transcripts, enzyme activities and metabolites in arabidopsis rosettes to a progressive decrease of temperature in the non-freezing range. *Plant Cell and Environ*. 2003; 31: 518–547.
20. Wallidano TC, Boget N, Claporals I, Santos M, Torne JM. Polyamine and free amino acid variations in NaCl-treated embryogenic maize callus from sensitive and resistant cultivars. *J. Plant physiol*. 1996; 149: 179-185.
21. Wang W. Vinocur XB, Shoseyov O, Altman A. Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. *Acta Hort.* 2001;560: 285-292.
22. Wang W, Vinocur B, Altman A. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*. 2003; 218: 1–14.
23. Yancey P. Compatible H, Counteracting S. Cellular and molecular physiology of cell volume regulation. Ed. K Strange. Austin, TX: CRC Press. 1994. pp. 81-109.