International Journal of Research in Agriculture and Forestry
Volume 5, Issue 9, 2018, PP 21-30
ISSN 2394-5907 (Print) & ISSN 2394-5915 (Online)

Metabolite Analysis and their Quantitative Variation in Healthy and Pest Infested Solanum melongena L. Using NMR Spectroscopy

Hema Bisht[1*], M. K. Bhatnagar[1], Prakhar Bhatnagar[2]and Arun K Murthy[3]

[1]Department of Chemistry, Pt. Shambhu Nath Shukla University, Shahdol, MP 484001, India
[2]Maa Bharti Senior Secondary School, Kota, Rajasthan 324005, India
[3]NMR Research Centre, Indian Institute of Science (IISc), Bengaluru, Karnataka 560012, India

***Corresponding Author:** Hema Bisht Department of Chemistry, Pt. Shambhu Nath Shukla Government P. G. College, Shahdol, MP 484001, India.*

ABSTRACT

The present study deals with metabolites variation analysis in healthy and pest infested Solanum melongena L. using NMR spectroscopy technique. Total forty-one metabolites were identified in which twenty two metabolites were quantified in leaf while twenty-one metabolites in stem. Discriminating metabolites were elucidated by diverse 2D-NMR (TOCSY) technique after sorting out different significant signals using 1H NMR measurements. Among the elucidated metabolites carboxylic acids, amino acids, carbohydrates, sugar alcohols, nucleotides, acetone, methylamine, propylene glycol, trimethylamine and adenine were found to be the contributing to the differentiation between healthy and infested (leaves and stems) S. melongena. Highly significant qualitative and quantitative differences were noticed between the leaf and stem samples. This change in metabolite profile during healthy and pest infested represents the change in metabolomic fluxes in different pathways.

Keywords: *Solanum melongena, H. armigera, metabolites, pest infestation, NMR, TOCSY.*

INTRODUCTION

Solanum melongena L. commonly known as eggplant, aubergine, guinea squash or brinjal is an economically important vegetable crop of tropical and temperate parts of the world. Its fruits are quite high in nutritive value and comparable with tomato as a good source of vitamins, dietary fiber and minerals (particularly iron) (Salunkhe and Kadam 1998). It is the most widely consumed fruits with annual production over 150 million metric tons (Laura Perez-Fons et al., 2014). It has been medicinal values also, such as, tissue extracts have been used for treatment of asthma, bronchitis, cholera, and dysuria; fruits and leaves are beneficial in lowering blood cholesterol (Kashyap et al., 2002). Its production is severely affected by biotic and a biotic stresses.

Among biotic stresses, pest infestation is considered as a severe threat for plant growth and crop production (Paul W. Pare., 1999). In response to biotic stress, plants produce a diverse range of metabolites which play an important role in plant defence mechanism (Taiz & Zeiger., 2006) and it causes a re-allocation of resources to leaf and stem storage tissues which increases the plant's defence mechanism (Schultz J.C., 2013). There are reports in the metabolites, indicating a rapid and significant plant response due to herbivory damage (Xiao Ming Cai et al., 2013; Ming gang Wang et al., 2014; U. Niinemets et al., 2013; Amy M. 2013). The most serious and widely distributed polyphagous insect pest is Helicoverpa armigera Hübner (Lepidoptera: Noctuidae). Its larvae stage causes severe damage to the reproductive and vegetative tissues of economically important crops including egg plant, tomato, cotton, chickpea, pigeonpea, tobacco, maize, sorghum, wheat, groundnut, sunflower and chillies etc (Gowda et al., 2005). The studies on variation of metabolites in connection to plant-pathogen interaction have been reported by NMR technique (Lima et al., 2010; Bollina et al., 2010; Ward et al., 2010; Jones et al., 2011).

The primary and secondary metabolites play an important role in plant survival (Zhao TJ et al., 2007) in defence mechanism against biotic and abiotic stresses (Williams RJ et al., 2004). So, it is imperative to study the variation of the

metabolites in connection to plant-pest interaction. This will help in better understanding of metabolic responses from S. melongena L. during pest infestation.

Material And Method

Egg plants (S. melongena BCB-11) seeds were sown in trays (52 cm x 27 cm) placed in a cultivation chamber at 24℃. Later, the seedlings were transplanted into pots. On fully matured brinjal plants (after 60 days), the larvae of H. armigera two per plant was inoculated. After one month, the egg plants were completely infested by larvae. The leaves and stems were collected after 30 days of the inoculation for the extraction process. Dried samples of 3 g each leaves and stems were taken for extraction by hexane (1:10 w/v). The solvent portion were collected by filtration and repeated five times until the hexane layers to become almost colourless. The separated solvent layer was concentrated under reduced pressure. The resulting hexane extracted sticky mass was stored at -5ºC and it was further used for derivatization prior to GC-MS analysis. After filtration the remaining solid plant material (residue) was further extracted by 80:20 of methanol: water solvent system. Methanol-water extracts was defatted with hexane. Defatted water-methanol layers were concentrated under reduced pressure. The samples were then lyophilized to dryness and the resulting solid was again stored at -80 ºC for further NMR analyses.

All NMR experiments were carried out at 25°C unless otherwise stated. The known quantity of samples were dissolved in 500 µl of D2O containing 0.81µM TSP (99.9% Sigma-Aldrich) for NMR analysis. The 1H NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (Bruker Biospin AG, Fallenden, Switzerland) equipped with 5 mm Broadband Observe (BBO) probe equipped with Z-shielded gradient, in a 5 mm NMR tube (Wilmad no. 07; Wilmad Labglass Buena, NJ, USA). 0.81µM TSP in deuterium oxide was inserted into the NMR tube before recording the spectra. TSP served as chemical shift reference as well as internal standard for quantitative estimation. One-dimensional 1H NMR experiments were performed using a CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence and suppressing the residual water signal by pre-saturation with 65,536 time-domain data points, a spectral width of 4807.692 Hz and 512 scans. The flip angle of radio-frequency pulse was 90° with the total relaxation delay of 3.7 s. Free-induction decays were multiplied by an exponential function with a line broadening of 0.30 Hz, prior to Fourier transformation. The quantification of metabolites was carried out taking into account the integrated area of NMR signals, molecular weight and number of protons of both TSP and metabolite.

To confirm the assignments of the metabolites, one-dimensional (1D) 1H NMR and two-dimensional (2D) total correlation spectroscopy (TOCSY) were carried out using the Bruker's standard pulse program library. In TOCSY, the spin locking time was set to 80 ms, which included duration of 2.5 ms for the trim pulses. For each t1, 40 transients were added with 2048 complex data points. The data were weighted with 90°shifted squared sine window function in F1 and F2 dimensions before double Fourier. Evaluation version of Chenomx Metabolite profiler software (version 7.6) (Edmonton, Alberta, Canada) was used to confirm the assignments.

All the chemical reagents were of analytical grade (Mallinckrodt Baker France, Noisy-Le-Sec, France). D2O (99.9%) was purchased from Cambridge Isotopic Laboratories (Andover, MA, USA), TSP (98%) from Aldrich (Saint-Quentin Fallavier, France).

Quantification Of Polar Metabolites

Identified polar metabolites were quantified by integrating the distinct signals of each metabolite with respect to the intensity of the nine protons of TSP (in D2O, 0.375% w/v) on the dry weight basis of leaves and stems using the following equation (Diehl et al., 2007).

Weight of metabolite = Integral value of (metabolite)/ Integral value of (TSP)* Molecular wt (metabolite)/Molecular wt (TSP)*Number of Protons in TSP/Number of Protons in metabolite*wt of TSP

Results

Different class of metabolites, such as amino acids, carbohydrates, sugars, sugar alcohols and organic acids, were detected in the 1H NMR spectra of the methanol/water (80:20) extract. Total forty-one chemically diverse metabolites were identified, in which twenty two metabolites were quantified both in leaf and stem. The entire 1H spectrum of aqueous fraction may be divided into three major

regions. (0.0-3.5 ppm) region is rich with amino acids. δ (3.5-5.5 ppm) contains sugars and rest of the spectrum from δ (5.6-10.0 ppm) is dominated by aromatic compounds .

The 1H NMR spectra showed signals of leucine, isoleucine, valine, asparagine, citrate, ornithine, γ-aminobutyric acid (GABA), malic acid, succinic acid, ketoglutaric acid (KGA), acetone, α-linolenic acid, sarcosine, methylamine, propyleneglycol, trimethylamine in the high-field region (0.0-3.0 ppm). In the mid-field region (3.0-5.5 ppm) resonance assigned were glucose (Glc), sucrose, choline, fructose, glycerol, phenylamine and fucose. While in the downfield region (5.6-10.0 ppm), metabolites identified were formate, trigonelline, maleic acid, fumarate, tyrosine, uridine, chlorogenic acid, cytidine, adenine and guanosine. The 1H NMR chemical shifts along with its qualitative variability of identified metabolites are listed in Table 1.

The 1H NMR spectral complexity (overlapping signals) did not allow quantification of all the metabolites. However, 22 metabolites in leaf while 21 metabolites in stem were quantified by integrating the distinct characteristic signals of each metabolite with respect to the intensity of the nine protons of TSP (in D2O, 0.375%, w/v) on the dry weight (Diehl and Holzgrabe, 2007; Sidhu et al., 2011). The concentration of all the metabolites varied significantly between healthy and pest infested brinjal leaves and stems. NMR spectra of aqueous extracts were analyzed by using a combination of 1D and 2D NMR experiments. The identification of metabolites was further validated by comparing the 1H spectra of reference compounds with the existing literature values (Bharti et al., 2011; Chatterjee et al., 2010; Gil et al., 2000; Jayaprakasam et al., 2004; Matsuda et al., 2001; Sobolev et al., 2005). The reference compounds in the Biological Magnetic Resonance Data Bank (BMRB) were also used for characterizing the metabolites (Markley et al., 2007). The stack-plot of 1H NMR spectra of healthy and pest infested leaf and stem showed signal intensities of the identified metabolites present in the healthy and pest infested leaves. The assignments were confirmed by 1D proton NMR and 2D TOCSY spectrum of aqueous extract. The concentration of metabolites varied significantly in the healthy and pest infested leaves and stems (Table 2 and Table3). The stack-plot of 1H NMR spectra of aqueous extracts of all the developmental stages showed signal intensities of the identified metabolites present in the leaves. The resonances were confirmed by the use of the 1H TOCSY spectrum of aqueous extracts. Table 1 summarizes all compounds identified in 1H NMR spectra with the chemical shifts and the coupling constants of the detected signals.

Leaf Metabolite Contents

The polar extract of brinjal leaves mainly contains carboxylic acids, amino acids, sugars, sugar alcohol, nucleoside, and some other class of metabolites like acetone, methylamine, propylene glycol, trimethylamine, adenine and trimethylamine N- oxide (TMAO). (Table 2)

Carboxylic Acids And Its Derivatives

Carboxylic acids are important metabolites in brinjal leaves and they are necessary for development of brinjal plant organs and coordination of growth. Total 11 carboxylic acids were detected in polar leaf extract. Out of eleven carboxylic acids, four were quantified. The higher concentration of lactate (8.48±1.29) mg/gm, 2-hydroxyisobutyrate (1.23±0.18), acetate (2.21±0.33) and succinic acid (4.05±0.61) mg/gm of dry weight was detected in pest infested leaf.

Amino Acids Contents

Total 10 amino acids were detected in polar leaf extract. Out of ten amino acids, six metabolites were quantified. The higher concentration of alanine (12.31±1.87) mg/gm, leucine (3.49±0.53), tyrosine (3.00±0.45), valine (6.86±1.04), isoleucine (3.47±0.52)mg/gm of dry weight was detected in pest infested leaf while phenylalanine (3.62±0.55) mg/gm were present in pest infested leaf, not detected in healthy leaf.

Sugars And Sugar Alcohol Contents

Four sugar metabolites were detected (glucose, fructose, fucose and sucrose) in polar extract of leaf. Among them three metabolites were quantified. The higher concentration of glucose (3.08±0.46), sucrose (35.10±5.33) and fructose (69.01±10.49) mg/gm of dry weight was in pest infested leaf. Sugar alcohols like myoinositol and scyloinositol were detected in polar leaf extract among them myoinositol was quantified and was higher (115.63±17.58) mg/gm of dry weight in pest infested leaf.

Nucleoside Contents

Two nucleosides (uridine and cytidine) were detected and quantified. Uridine concentration (2.14±0.32) was higher in pest infested leaf, while cytidine (0.97±0.14) mg/gm was present in pest infested leaf.

Other Metabolites Contents

Other metabolites concentration like acetone (0.24±0.03) and methylamine (0.43± 0.06 mg/gm of dry weight were present in pest infested leaf.

Stem Metabolite Contents

Carboxylic Acids And Its Derivatives

Total 10 carboxylic acids were detected in polar stem extract. Out of ten carboxylic acids, six metabolites were quantified. The higher amount of lactate (11.39±2.81), 2-hydroxyisobutyrate (0.67±0.16), succinic acid (1.54±0.38), acetate (1.14±0.28) and GABA (8.15±2.01) mg/gm of dry weight were found in healthy stem. (Table 3)

Amino Acids Contents

Total 10 amino acids were detected in polar stem extract. Out of ten amino acids, six metabolites were quantified. The higher concentration of alanine (5.90±0.44) mg/gm, valine (3.60±0.27), phenylalanine (3.31±0.24), sarcosine content (0.34±0.02), tyrosine (1.64±0.12) and isoleucine (2.02±0.15) mg/ gm of dry weight were detected in pest infested stem.

Sugars and sugar alcohol contents

Major three sugar metabolites (glucose, sucrose and fructose) were detected and quantified in polar extract of stem. Among these polar metabolites higher concentration of sucrose (54.63±13.50), glucose (20.91±5.16) and fructose (46.39±11.46) mg/ gm was present in healthy stems. Sugar alcohol, myoinositol and scyloinositol were detected. The higher amount of myoinositol was (67.22±16.61) mg/gm of dry weight in healthy stem.

Table1. *Chemical shift of 1H-NMR identified metabolites of leaves and stem samples from healthy and pest infested S. melongena.*

Sl.No.	Metabolites	Chemical Shift (ppm)	Multiplicity	Assignment
1.	Leucine	0.95 3.50	t m	CH_3 CH_2
2.	Isoleucine	1.01 1.24 1.94	d m m	CH_3 CH_2 CH
3.	Valine	0.98 1.04 2.27 3.60	d d m d	CH_3 CH_3 CH CH
4.	Glucose	3.64 4.58 5.23	d d d	CH CH CH
5.	Sucrose	4.05 4.17 5.41	t d d	CH CH CH
6.	Fructose	3.70 3.71 4.23	m m d	CH CH CH
7.	Asparagine	2.93 4.06	m m	CH_2 CH
8.	Citrate	2.53 2.71	d m	$1/2CH_2$ $1/2CH_2$
9.	Ornithine	1.98 3.06	s t	CH_2 CH_2
10.	GABA	1.89 2.33 3.0	m t t	CH2 CH2 CH2
11.	Lactate	1.33 4.12	d q	CH_3 CH
12.	Alanine	1.48 3.73	d m	CH_3 CH

13.	Myoinositol	3.28 3.56 4.05	t dd t	CH CH CH
14.	Formate	8.46	s	CH
15.	Trigonilline	8.01 8.85 9.16	s m s	CH CH CH
16.	Acetate	1.97	s	CH_3
17.	Malic acid	2.62 4.34 2.37	m dd dd	CH_2 CH CH_2
18.	Succinic acid	2.43	s	CH_2
19.	Choline	3.20	s	$+N\text{-}Me_3$
20.	Glycerol	3.59 3.65	d m	CH_2 CH
21.	Phenylalanine	3.11 3.96 7.28	m dd m	CH2 CH CH
22.	Maleic acid	5.80	d	-CH=CH
23.	Fucose	1.24 5.20	m d	CH CH
24.	Fumarate	6.52	s	CH
25.	Tyrosine	6.93 7.20	d d	CH CH
26.	KGA	2.47 3.0	t t	CH_2 CH_2
27.	Uridine	5.94 7.94	d d	-CH=CH- -CH=CH-
28.	Acetone	2.01	s	CH_3
29.	Scylo inositol	3.23	s	CH
30.	Chlorogenic acid	5.97 7.95 5.61	m d m	CH CH CH
31.	α- Linolenic acid	0.96 1.31	t t	CH3 -CH2-
32.	Hydroxyisobutyrate	1.37	s	$(CH_3)_2$
33.	Sarcosine	2.73	s	CH3
34.	Methylamine	2.6	s	CH3
35.	Cytidine	6.06	d	CH
36.	Propylene glycol	1.01	d	CH2
37.	Trimethylamine	2.9	s	$(CH_3)_3$
38.	Asparagine	2.93 4.06	m m	CH3
39.	Trimethylamine-N-oxide (TMAO)	3.26	s	$(CH_3)_3$
40.	Adenine	8.26	s	CH
41.	Guanosine	8.0	s	CH

Table2. *Quantitative variability in NMR analyzed metabolites from aqueous extracts of healthy and pest infested leaf samples of S. melongena. Three numbers of replicates were taken in each group of different parts. Mean values ± SD of mg/gm of the dry weight of leaf sample, ND = not detected.*

Sl.No.	Metabolites	Healthy leaf	Pest infested leaf
1.	Lactate	0.55±0.26	8.48±1.29
2.	Alanine	0.51±0.24	12.31±1.87
3.	Valine	0.53±0.25	6.86±1.04
4.	2-Hydroxyisobutyrate	0.09±0.04	1.23±0.18
5.	Acetate	0.09±0.04	2.21±0.33
6.	GABA	0.57±0.27	15.08±2.29
7.	Succinic acid	0.06±0.03	4.05±0.61
8.	Sarcosine	0.04±0.02	1.65±0.25
9.	Choline	0.37±0.17	11.16±1.69

10.	Trigonelline	0.02±0.01	5.33±0.81
11.	Isoleucine	0.21±0.10	3.47±0.52
12.	Leucine	0.32±0.15	3.49±0.53
13.	Acetone	0.07±0.03	0.24±0.03
14.	Methylamine	0.01±0.00	0.43±0.06
15.	Uridine	0.02±0.01	2.14±0.32
16.	Phenylalanine	ND	3.62±0.55
17.	Tyrosine	0.19±0.09	3.00±0.45
18.	Cystidine	ND	0.97±0.14
19.	Glucose	0.03±0.01	3.08±0.46
20.	Sucrose	0.90±0.43	35.10±5.33
21.	Fructose	1.73±0.82	69.01±10.49
22.	Myoinositol	4.38±2.08	115.63±17.58

Table3. *Quantitative variability in NMR analyzed metabolites from aqueous extracts of healthy and pest infested stem samples of S. melongena. Three numbers of replicates were taken in each group of different parts. Mean values ± SD of mg/gm of the dry weight of stem sample, ND = not detected.*

Sl.No.	Metabolites	Healthy stem	Pest infested stem
1.	Lactate	11.39±2.81	8.05±0.60
2.	Alanine	5.86±1.44	5.90±0.44
3.	Valine	3.37±0.83	3.60±0.27
4.	2-Hydroxyisobutyrate	0.67±0.16	0.65±0.04
5.	Acetate	1.14±0.28	1.09±0.08
6.	GABA	8.15±2.01	6.91±0.51
7.	Succinic acid	1.54±0.38	0.67± 0.05
8.	Sarcosine	0.26±0.06	0.34±0.02
9.	Choline	13.29± 3.28	8.95±0.67
10.	Fumaric acid	0.14±0.03	0.67±0.05
11.	Trigonelline	3.62±0.89	0.67±0.05
12.	Isoleucine	1.24±0.30	2.02±0.15
13.	Leucine	3.77±0.93	2.27±0.17
14.	Uridine	7.18±1.77	0.71±0.05
15.	Phenylalanine	0.62±0.15	3.31±0.24
16.	Tyrosine	0.44±0.11	1.64±0.12
17.	Cystidine	3.96±0.98	0.06±0.004
18.	Glucose	20.91±5.16	11.33±0.84
19.	Sucrose	54.63±13.50	51.77±3.87
20.	Fructose	46.39±11.46	39.95±2.98
21.	Myoinositol	67.22±16.61	44.38±3.32

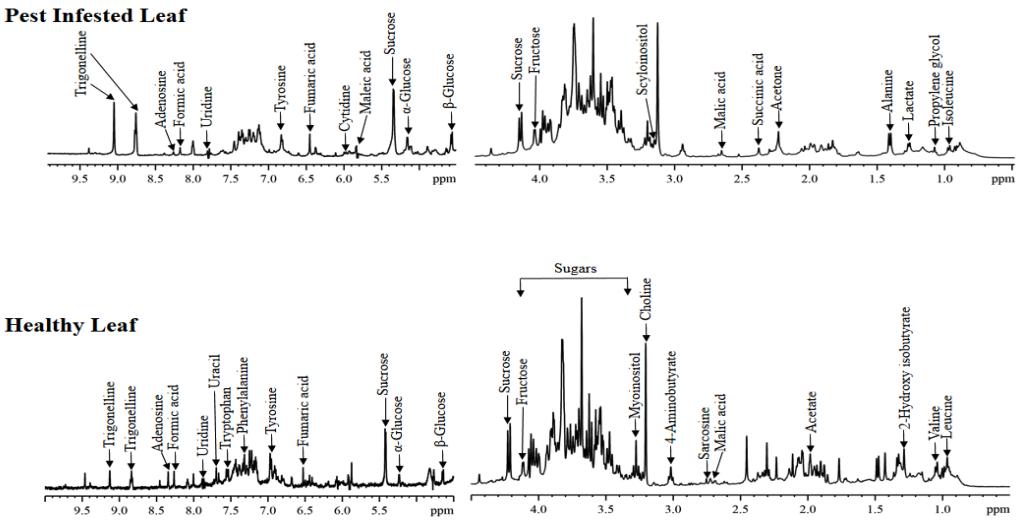


Figure1. *1H NMR spectra of polar extracts of healthy and pest infested leaf of S. melongena.*

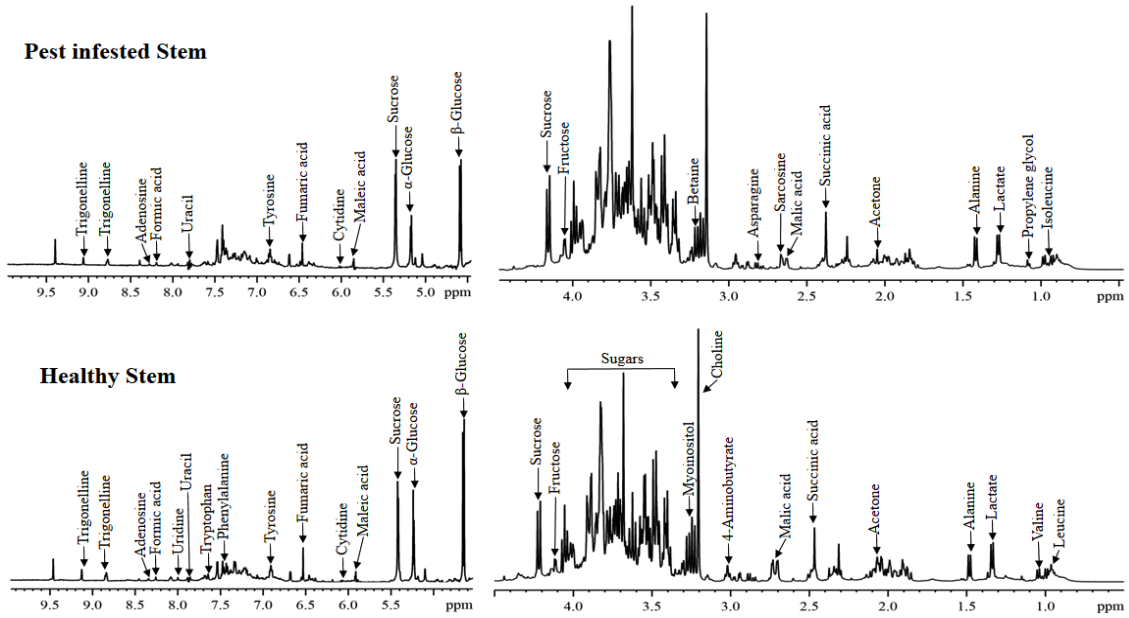
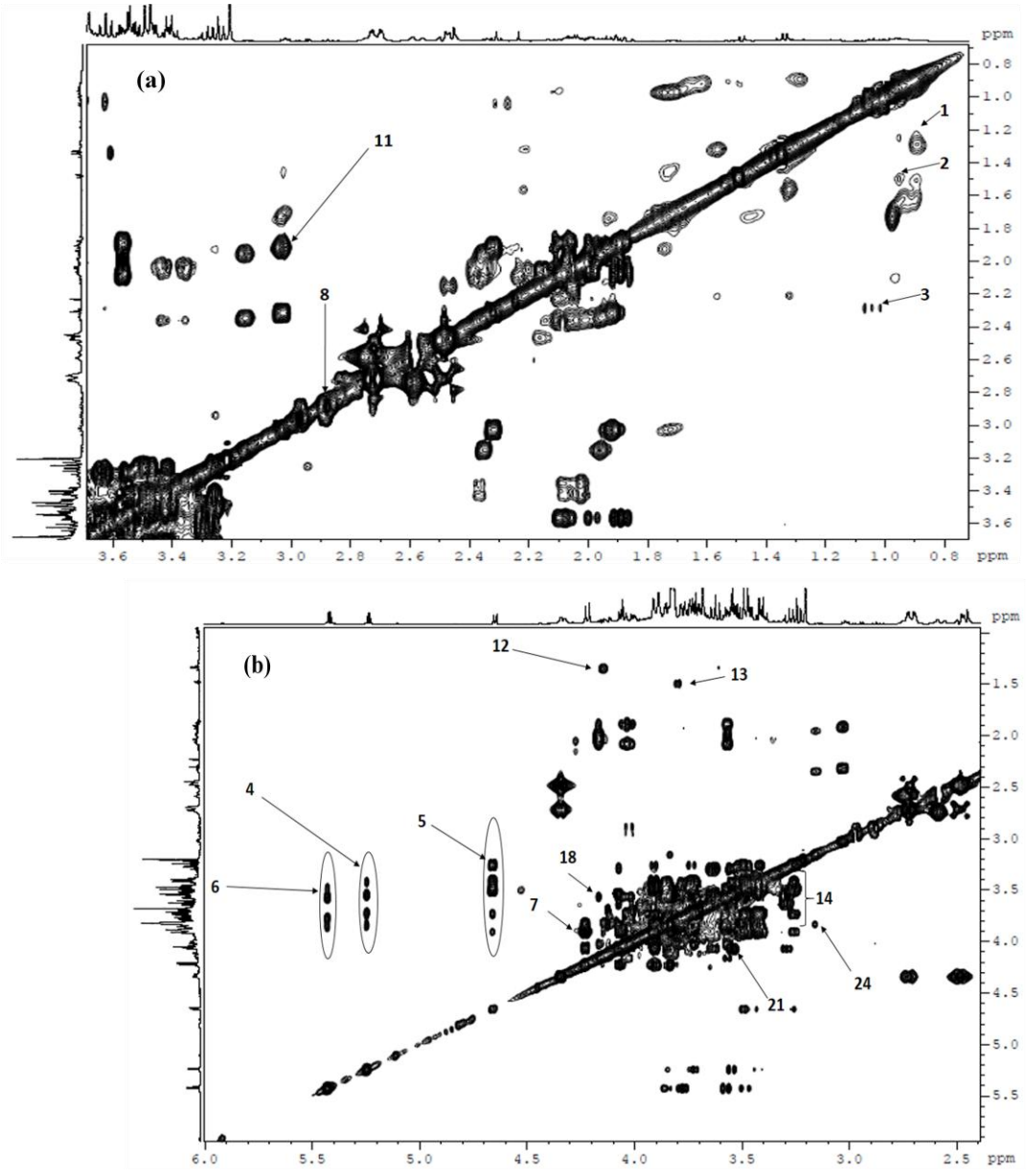


Figure2. *1H NMR spectra of polar extracts of healthy and pest infested stem of S. melongena.*



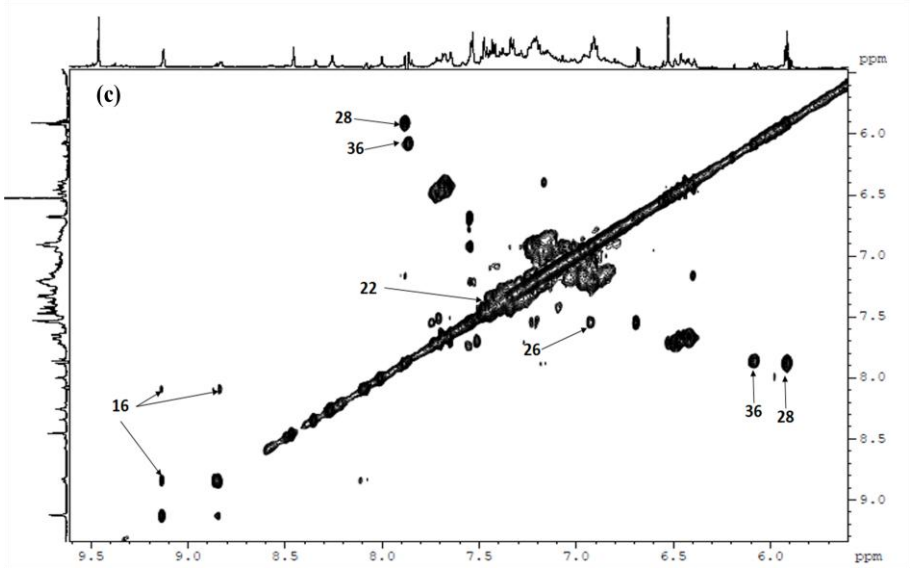


Figure3. *Two-dimensional homonuclear 1H-1H experiment (TOCSY) of S. melongena recorded in (a) high field region (δ 0.7- δ 3.0), (b) mid field region (δ 3.0- δ 5.6) and (c) low field region (δ 5.6- δ 10) spectra.*

DISCUSSIONS

Myo-inositol is a sugar-like carbohydrate produced by most plants and is important for phosphate storage, cell wall biosynthesis, the production of stress related molecules, cell-to-cell communication, storage and transport of plant hormone (Loewus, F. and Loewus, M.W. 1983). In our study myoinositol content was higher 115.63±17.58 mg/gm of dry weight in pest infested leaves.

In plant GABA production is activated cytosolic acidification mechanism. This mechanisms is known to accompany biotic and abiotic stresses (Kinnersley and Turano, 2000; Fait et al., 2007). In our study GABA and alanine were detected higher in pest infested leaf. Similar types of observations were reported due to plant response to several types of stress (Wallace et al., 1984; Mayer et al., 1990; Monlise et al., 2003; Kato-Noguchi and Ohashi, 2006; Miyashita et al., 2007; Allan et al., 2008). Accumulation of alanine and GABA metabolites during stress, contributes to protein and membrane stabilization as well as to maintaining osmotic pressure reported by Kaplan et al., 2004.

The concentration of glucose, sucrose and fructose sugars were higher in pest infested leaf. It was well reported that soluble sugars such as glucose and sucrose help regulate many development and physiological processes in plants (Koch, 1996; Smeekens, 1997; Sheen et al., 1999). Sugars were also thought to control photosynthesis (Krapp et al., 1993), flowering (Bernier et al., 1993) and starch synthesis (Koch, 1996).

CONCLUSION

The results of the present study show that the metabolites such as changes amino acids, organic acids, sugars and sugar alcohols. During plant development and growth the chemical composition of the plant changes. This change in metabolite profile during healthy and pest infested leaf and stem represents the change in metabolomic fluxes in different pathways. 1H NMR spectroscopy used as a valuable tool for unbiased metabolite profiling of healthy and pest infested leaves and stem of S. melongena.

ACKNOWLEDGEMENT

Author is thankful for Indian Institute of Sciences (IISc) Bengaluru for providing NMR facility.

REFERENCES

[1] D.K. Salunkhe, S.S. Kadam. (1998).Handbook of vegetable science and technology: production, composition, storage, and processing

[2] Laura Perez-Fons, Tom Wells, Delia I. Corol, Jane L. Ward, Christopher Gerrish, Michael H. Beale, Graham B. Seymour, Peter M. Bramley and Paul D. Fraser.(2014). Sci Rep. A genome-wide metabolomic resource for tomato fruit from *Solanum pennellii*; 4: 3859.

[3] Kashyap, Anil K, Raghuram Rajan, and Jeremy C. Stein. (2002). Banks as liquidity providers: An explanation for the co-existence of lending and deposit-taking, Journal of Finance57:33-73.

[4] PW Paré, JH Tumlinson. (1999). Plant volatiles as a defense against insect herbivores -Plant physiology, Am Soc Plant Biology.

[5] L Taiz, E Zeiger. (2006). Fisiologia vegetal.

[6] Jack C. Schultz, Heidi M. Appel, Abigail P. Ferrieri and Thomas M. Arnold Front. (2013). Flexible resource allocation during plant defense responses. Plant Science.

[7] Xiao-ming Cai, Xiao-Ling Sun, Wen-Xia Dong, Guo-chang Wang, Zong-mao.(2013). Chen Chemoecology. Herbivore species, infestation time, and herbivore density affect induced volatiles in tea plants

[8] Wang K, *et al*. (2014) Suitability of anaerobic digestion effluent as process water for corn fuel ethanol fermentation. Water Sci Technol 69(9):1894-9.

[9] Jack C. Schultz, Heidi M. Appel, Abigail P. Ferrieri and Thomas M. Arnold Front. (2013). Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage, Plant Science.

[10] M. Trowbridge, Paul C. Stoy BVOC-Mediated Plant-Herbivore Interactions Amy Biology, Controls and Models of Tree Volatile Organic Compound Emissions pp 21-46.

[11] Gowda, B. T., Shetty, M. & Jayalakshmi, K. L. (2005). Z. Naturforsch. Teil A, 60, 106-112.

[12] Zhao C, Li Z, Yan B, Harrison TJ, Guo X, *et al*., (2007) Comparison of real-time fluorescent RT-PCR and conventional RT-PCR for the detection of hepatitis E virus genotypes prevalent in China. Journal of medical virology 79: 1966-1973.

[13] Williams,R.J., and Martinez, N.D (2004).Limits to tropic levels and omnivory in complex food webs: Theory and data, Am.Nat .163,458-468.

[14] Lima MR, Felgueiras ML, Graca G, Rodrigues JE, Barros A, Gil AM, *et al* (2010) . NMR metabolomics of Esca disease-affected Vitis vinifera cv. Alvarinho leaves. J Exp Bot; 61:4033-42.

[15] Bollina V, Kumaraswamy GK, Kushalappa AC *et al*., (2010). Mass spectrometry-based metabolomics application to identify quantitative resistance-related metabolites in barley against fusarium head blight. Molecular Plant Pathology 11, 769-82.

[16] Ward JL, Forcat S, Beckmann M, Bennett M, Miller SJ, Baker JM, *et al* (2010*)*. The metabolic transition during disease following infection of Arabidopsis thaliana by Pseudomonas syringae pv. tomato. Plant J; 63: 443-57.

[17] Jones OA, Maguire ML, Griffin JL, Jung YH, Shibato J, Rakwal R, *et al*(2011). Using metabolic profiling to assess plant-pathogen interactions: an example using rice (Oryza sativa) and the blast pathogen Magnaporthe grisea. Eur J Plant Pathol; 129:539-54.

[18] Bharti, S.K., Bhatia, A., Tewari, S.K., Sidhu, O.P., Roy, R. (2011). Application of HR-MAS NMR spectroscopy for studying chemotype variations of Withania somnifera (L.) Dunal. Magnetic Resonance in Chemistry 49, 659-667.

[19] Chatterjee, S., Srivastava, S., Khalid, A., Singh, N., Sangwan, R.S., Sidhu, O.P., Roy, R., Khetrapal, C.L., Tuli, R. (2010). Comprehensive metabolic fingerprinting of Withania somnifera leaf and root extracts. Phytochemistry 71, 1085-1094.

[20] Gil, A.M., Duarte, I.F., Delgadillo, I., Colquhoun, I.J., Casuscelli, F., Humpfer, E., Spraul, M., (2000). Study of the compositional changes of mango during ripening by use of nuclear magnetic resonance spectroscopy. Journal of Agricultural and Food Chemistry 48, 1524-1536.

[21] Jayaprakasam, B., Fitch, W., Vareed, S.K., Nair, M.G. (2012). Identification of metabolites in Withania sominfera fruits by liquid chromatography and high resolution mass spectrometry. Rapid Communications in Mass Spectrometry 26, 1277-1290.

[22] Matsuda, H., Murakami, T., Kishi, A., Yoshikawa, M. (2001). Structures of withanosides I, II, III, IV, V, VI, and VII, new withanolide glycosides, from the roots of Indian Withania somnifera DUNAL, and inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum. Bioorganic and Medicinal Chemistry 9, 1499-1507.

[23] Sobolev, A.P., Brosio, E., Gianferri, R., Segre, A.L. (2005). Metabolic profile of lettuce leaves by high-field NMR spectra. Magnetic Resonance in Chemistry 43, 625- 638.

[24] Markley, J.L., Anderson, M.E., Cui, Q., Eghbalnia, H.R., Lewis, I.A., Hegeman, A.D., Li, J., Schulte, C.F., Sussman, M.R., Westler, W.M., Ulrich, E.L., Zolnai, Z. (2007). New bioinformatics resources for metabolomics. Pacific Symposium on Biocomputing 12, 157.

[25] Frank A. Loewus and Mary W.Loewus. (1983). myo-Inositol: Its Biosynthesis and Metabolism.Ann. Rev. Plant Physiol., 34: 137-61.

[26] AM Kinnersley, FJ Turano. (2000). Critical Reviews in Plant Sciences, Taylor & Francis Gamma aminobutyric acid (GABA) and plant responses to stress.

[27] Fait A, Fromm H, Walter D, Galili G, Fernie AR. (2007). Highway or byway: the metabolic role of the GABA shunt in plants. Trends in Plant Science 13, 14-19.

[28] Wallace W, Secor J, Schrader LE. (1984). Rapid accumulation of γ-aminobutyric acid and

alanine in soybean leaves in response to an abrupt transfer to lower temperature, darkness, or mechanical manipulation. Plant Physiology 75, 170-175.

[29] Mayer RR, Cherry JH, Rhodes D. (1990). Effects of heat shock on amino acid metabolism of cowpea cells. Plant Physiology 94, 796-810.

[30] Monlise EB-I, Parola AH, Kost D. (2003). Low-frequency electromagnetic fields induce a stress effect upon higher plants, as evident by the universal stress signal, alanine. Biochemical and Biophysical Research Communications 302, 427-434.

[31] Kato-Noguchi H, Ohashi C. (2006). Effects of anoxia on amino acid levels in rice coleoptiles. Plant Production Science 9, 383-387

[32] Miyashita Y, Dolferus R, Ismond KP, Good AG. (2007). Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in Arabidopsis thaliana. The Plant Journal 49, 1108-1121.

[33] Allan WL, Simpson JP, Clark SM, Shelp BJ. (2008). γ-Hydroxybutyrate accumulation in Arabidopsis and tobacco plants is a general response to abiotic stress: putative regulation by redox balance and glyoxylate reductase isoforms. Journal of Experimental Botany 59, 2555-2564.

[34] Kaplan F, Kopka J, Haskell DW, Zhao W, Schiller KC, Gatzke N, Sung DY, Guy CL. (2004). Exploring the temperature-stress metabolome of Arabidopsis. Plant Physiology 136, 4159-4168.

[35] Karen E. Koch Yong Wu Jian Xu.(1996). Sugar and metabolic regulation of genes for sucrose metabolism: potential influence of maize sucrose synthase and soluble invertase responses on carbon partitioning and sugar sensing Journal of Experimental Botany, Volume 47, , 1179-1185

[36] Smeekens,S. and Rook F.(1997) Sweet sensations: Sugars sensing and sugar- mediated signal transduction in plants. Plant Physiology, 115, 7-13.

[37] JenSheen, LiZhouJyun-ChyunJang. (1999). Sugars as signaling molecules, Current Opinion in Plant Biology Volume 2, Issue 5, 410-418.

[38] Krapp A, Hofmann B, Schäfer C, Stitt M. (1993). Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis? The Plant Journal 3, 817-828.

[39] G Bernier, A Havelange, C Houssa, A Petitjean, and P Lejeune. (1993). Physiological Signals That Induce Flowering. Plant Cell. Oct; 5(10): 1147-1155.

Citation: *Hema Bisht, M. K. Bhatnagar, Prakhar Bhatnagar and Arun K Murthy.(2018). "Metabolite Analysis and their Quantitative Variation in Healthy and Pest Infested Solanum melongena L. Using NMR Spectroscopy". International Journal of Research in Agriculture and Forestry, 5(9), pp.21-30.*