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Changes in volatile metabolites in leaves of *Vigna unguiculata* L. Walp. in response to Groundnut Bud Necrosis Virus infection

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ABSTRACT

Vigna unguiculata L. Walp. (Cowpea) is an important legume crop grown in arid and semiarid regions of Rajasthan. During the field observations, the crop was found infested by bud necrosis like disease. This study focuses on the effects of the Groundnut Bud Necrosis Virus on volatile metabolite changes in *Vigna unguiculata.* Methanolic extracts of healthy and virus-infected leaves of cowpea were used to study changes in metabolites using Gas Chromatography - Mass Spectrometry. In both samples, a differential accumulation of metabolites was noticed. A total of 133 metabolites having 42 types of functional groups were identified. 9 metabolite groups were common in both the samples. Among common metabolites, enhanced accumulation of alkanes, diterpenes, halogenated hydrocarbons and organofluorine was noticed while fatty acids, fatty acid esters, triterpenes, alcohols and ketones were reduced significantly in virus-infected samples. This study shows significant alteration in the volatile metabolite profile of cowpea leaves due to GBNV infection which may further be helpful to understand the mechanism of disease development.

Key words : *Vigna unguiculata,* Groundnut Bud Necrosis Virus, Gas Chromatography - Mass Spectrometry, volatile metabolite.

INTRODUCTION

Vigna unguiculata L. Walp. (Cowpea) is an essential legume crop. According to the annual report (2021-22) Government of India Ministry of Agriculture & Farmers Welfare, cowpea is cultivated by 37 countries with 10 % contribution in total pulse production. Its versatility, nutritional value, and adaptability to diverse agroecological zones make it a valuable crop for both food and feed purposes. The crop is cultivated mainly in the arid and semi-arid belt of Rajasthan, Punjab, Haryana, Delhi, and western Uttar Pradesh. Cowpea production is adversely affected by a number of pathogens such as bacteria, viruses and fungi. It is susceptible to at least 33 legume viruses from 16 genera and 11 families (Chatzivassiliou 2021). Orthotospovirus is a genus of plant viruses. Members of this genus are major constraints for vegetable and pulse crops in India. Groundnut bud necrosis virus is one of the important viruses of this group. GBNV belonging to the genus *Orthotospovirus,* family, *Tospoviridae*, is indigenous to the Indian subcontinent and has a wide natural host range (Jones, 2020)

It has been detected in 30 different crops and weed species belonging to eight families and

is the most prevalent and destructive *Orthotospovirus* in India (Basavaraj *et al*., 2017). In cowpea, GBNV has been reported first time by Rao *et al*., 1987.

Metabolite changes in biological systems due to pathogen attacks open the doors to understand the host-parasite interaction at biochemical levels. At the onset of viral infection and the progress of disease, plethora of changes in different biochemical pathways can occur. After viral infections, plants show frequent changes in photosynthesis-related metabolites (Dardick, 2007), and viral infection-induced alterations in carbohydrate metabolism which can be observed as changes in the levels of sugars (Kogovšek *et al*., 2016). It is also reported that the change in the production of amino acids after viral infection ultimately leads to successful defense responses by the affected plant (Srivastava *et al*., 2012) and also the success of virus replication (Bazzini *et al*., 2011). Plants also produce secondary metabolites after pathogen attack (Lopez-Gresa *et al*., 2012; Hanssen *et al*., 2011) and pathogenesis-related proteins, both of which are involved in plant defenses.

Various analytical methods are available to decode these changes in metabolites in biological systems, GC-MS among others is considered one of the most efficient, reproducible and well-used analytical methods except having a single inherent limitation that it can be applicable only for the separation and identification of volatile compounds having low molecular weights (50-600 Da). This method is also useful in identifying an array of biochemicals due to the availability of well-versed metabolite databases in the form of libraries (Beale *et al*., 2018). Prior to GC-Ms analysis, appropriate extraction of metabolites from targeted tissue enhances the efficacy of analysis. Many solvents have been used in literature for sample preparation for extraction of metabolites. It was earlier reported that methanol as an extraction solvent has high extraction efficiency over other solvents (Swamy *et al*., 2015, 2017). This research work aims to investigate changes in the volatile phytochemical profile of cowpea leaf samples upon GBNV infection growing in field conditions. The role of some of the important phytochemicals has been discussed.

MATERIALS AND METHODS

Experimental site and collection of plant material

In an open agricultural field of Tehsil Laxmangarh, Sikar district, Rajasthan (27.51° N, 75.16° E), a 150 sq. m plot was chosen for sowing cowpea seeds of the variety Pusa komal which were purchased from seed certified centre situated in IARI, Pusa campus, New Delhi. Seeds were shown in the last week of July 2021. Due to the prevalence of bud necrosis disease in neighbouring cowpea growing fields; symptoms of bud necrosis disease started to appear in experimental crops in early growth stages (within 15 days of seed showing). The confirmation of the causal pathogen of bud necrosis disease was made as Groundnut bud necrosis virus by RT-PCR amplification of the full-length nucleocapsid protein gene of GBNV using Tospo IV-N-Forward **(**5'ATGTCTAACGTC/TAAGCAA/GCTC3') and Reverse primers (5' TTACAATTCCA GCGAAGGACC3')**.** After full vegetative

growth of the crop (45 days old plants), leaves from disease-symptomatic (GBNV infected) and asymptomatic (healthy) plants were picked separately for further analysis.

Preparation of plant material and methanolic extraction for metabolite profiling

Virus-symptomatic and asymptomatic leaves picked from the experimental field were carried to the laboratory, and properly washed with distilled water. Extra water was removed using blotting papers and then both leave materials were shade dried separately at room temperature. After complete drying, leaves were ground to fine powder using an electric blender and the powder was stored in airtight containers at room temperature until further use. 10gms of powder, each of symptomatic and asymptomatic leaves was extracted in HPTLC grade methanol as extraction solvent using Soxhlet apparatus for almost 3 cycles of 6 hours (until the solvent in Soxhlet siphon became colourless). After complete extraction, the methanolic extract was filtered with Whatman no. 1 filter paper**,** concentrated up to dry at room temperature then weighed for yield (%) determination using the following formula-

%Yield of extract= Weight of dried extract(g)/Initial weight of dried plant material(g) ×100

After this, extracts were stored at 4°C in a refrigerator for further analysis. Before GC-MS analysis, a known concentration of the extract was prepared by redissolving dried extract in fresh methanol and then filtered through a 0.2-micron syringe filter. This filtered extract was used for volatile metabolite profiling using GC- MS.

GC-MS analysis

Phytometabolites analysis using GC-MS was performed to separate bioactive components present in methanolic extracts of both, virus-infected and healthy cowpea leaves. This experiment was performed using Gas Chromatography-Mass Spectrometry (QP2020 of Shimadzu, Japan) instrument which was equipped with a capillary column of Rxi 5 Sil MS (1,4-bis (dimethylsiloxy) phenyl dimethyl polysiloxane) of dimensions 30m, 0.25mm and 0.25µm. Helium (99.99%) was used as the carrier gas with a column flow of 1.04 mL/minute, linear velocity 37.1 cm/sec, purge flow 5 mL/min. at 56.9 kPa pressure with split ratio :15.0. 1µl volume of each sample was injected in the split mode. An electronic ionization system with70eV ionisation potential was used for GC-MS detection. Both the injector and ion source temperature were kept at 250^{0}C. At the beginning of the program, the oven temperature was kept at 5^{0}C for 2 min which was increased by a constant rate of 8^{0}C/min up to 200^{0}C and after 200^{0}C, it was increased by 10^{0}C/min up to 280^{0}C for 5 min. The mass scan range used was 40-600m/z with a 2000 scan speed. The total time of GC program was 33.75 minutes with 0.30 second scan interval.

Identification and analysis of metabolites:

The metabolites present in both extracts were identified by using mass spectra and the National Institute of Standards and Technology (NIST) library. The mass spectra of these samples were matched with the spectra of the known compounds available in NIST17. The relative per cent amount of each metabolite was determined by comparing its average peak area with the total area. Other

parameters like structure, molecular formulae, and molecular weight of these metabolites were also confirmed with the help of NIST. Metabolite classes for different compounds detected were assigned according to information available on PubChem (https://pubchem.ncbi.nlm.nih.gov/).

RESULTS AND DISCUSSION

For this study confirmation of GBNV was made by RT-PCR (Fig. 1). Clear visible symptoms of this virus such as chlorotic rings, necrotic rings and patches, venal necrosis, apical bud necrosis etc. have been reported from the study site which was in accordance with GBNV caused symptoms reported by Mandal et al 2012. Healthy and GBNV-infected cowpea leaf samples were collected from the field, dried and processed for extraction of metabolites. After methanolic extraction, a higher extraction yield was obtained from healthy leaves samples (10.48%) as compared to virus-infected leaves (8.6%). Dried extracts from each of the samples were redissolved in fresh methanol for preparation of 100ppm solution and processed for metabolite profiling by GC–MS.

GCMS analysis of methanolic extracts of GBNV infected cowpea leaves showed changes in volatile metabolite profile with reference to healthy plants (Table 3).

In total 133 metabolites were identified in both healthy (69) and virus infected leaves samples (64) on the basis of retention time indices, molecular mass and molecular formulas (Table 1& 2). These metabolites were broadly categorized into 42 classes according to the presence of specific functional groups in their chemical structure. In virus-infected leaves 64 metabolites were identified. These metabolites were assigned 17 classes including alkane (35), halogenated hydrocarbon (5), esterified acid (5), cyclohexenes (4), alcohols (2) and ether (2). In addition to these metabolites, 11 other metabolites each in a single were alkene, benzene derivative, Citronellyl linoleate, diterpene, triterpenoid, triterpene, fatty acid, fatty alcohol, fatty acid ester, ketone and organofluorine. On the other hand, in healthy leaves extracts, a total of 69 metabolites were detected (Table 1). These metabolites were assigned 34 classes including ketone (9), alcohols (5), fatty acid (5), alkanes (4), Carboxylic acids (4), fatty acid ester (4), benzofurans (3), sugars (3), pyrrolidine derivative (3), aldehyde (2), amide (2), Phenol (2), halogenated hydrocarbon (2) , and 21 metabolites were present in singles including alkane derivative, aziridine, carbonic acid ester, cyclic lactone, diazine, dioxolane, diterpene, diterpene alcohol, diterpenoid, ester, furanone, glycerol, glycoside, keto acid, organofluorine, phytosterol, steroid, triterpene, vaccenoate, valero lactone derivative and vitamin. In these 42 classes of detected metabolites, 9 metabolite classes were common in both the samples and 33 were different (Table 3). Among the different 33 classes, 25 classes were exclusively present in healthy leaves and 8 were exclusively in virus-infected leaves. It was found that among the commonly expressing metabolite classes; alkanes, diterpenes, halogenated hydrocarbons and organofluorine were upregulated while; alcohols, fatty acids, fatty acid esters, ketones and triterpenes were downregulated (Table 3) in virus-infected leaves as compared to healthy leaves. In

terms of particular compounds, in both samples, most of the compounds were unique except seven compounds known as Dodecane-4,6-dimethyl, 3-Ethyl-2,6,10-trimethylundecane, Heptadecane-2-methyl, n-Hexadecanoic acid, Tetratetracontane, Hexadecanoic acid-2-hydroxy-1-(hydroxymethyl)ethyl ester and Squalene which were present in both the samples.

Level of squalene, a triterpene compound detected in good quantity in HL (3.49%) showed around 50-fold reduction in VIL (0.07%) and associated phytosterols especially of gamma sitosterol (5.26% in HL) were not even detected in VIL of cowpea plants. This reduction can be correlated with another study in which virus-induced gene silencing of squalene synthase (responsible for precursor formation for triterpenoid) resulted in reduced squalene synthesis which led in a significant reduction in phytosterol production. It is possible that for successful disease development, GBNV may silence some key genes that regulate metabolic homeostasis to maintain the integrity and healthy state of cells (Singh *et al*., 2015).

Phytol, an acyclic diterpenoid compound was detected in HL samples (2.82%) but was absent in VIL samples, which is in accordance with another study in which Tomato yellow leaf curl virus disease in tomato resulted in a significant reduction in phytol content (Soliman *et al*., 2018).

On the other hand, diterpene compounds increased by 1.03-fold in VIL as compared to HL. Antiviral activities of diterpene were reported by Ma *et al*., 2016. This suggests that infected plants try to induce defense by producing compounds of antiviral potential.

Alcohols were in higher amounts in healthy leaves (2.86%) as compared to diseased (1.27%), carboxylic acids were detected only in healthy samples (1.16%), while long-chain alkanes and their derivatives (halogenated hydrocarbons) showed a significant increase in VIL (32.91%) as compared to HL (1.5%). In a study, it was found that compounds present in plant surface waxes such as long-chain alkane, carboxylic acid, and alcohols have their role in insect feeding stimulations (Viladomat and Bastida, 2015). The role of wax in whitefly interaction to cotton plants and transmission of Cotton leaf curl virus transmission has been reported by Ali et al., 2021. The differential expression of these metabolites in both leaf samples of cowpea may also change the feeding choices of the vector (thrips) for successful transmission of GBNV. This is also supported by another study in which heneicosane, one of the alkanes also reported in this study in VIL, has semiochemical-like properties and is reported as a mosquito attractant (Majumder *et al*., 2020). Sugar content was detected in HL in a good amount (13.86%) while not detected in VIL. It was earlier reported that *Ageratum enation virus* infection significantly affects the plant's primary metabolism resulting in decreased glucose and sucrose content in *Amaranthus hypochondriacus* L (Srivastava *et al*., 2012). It is suggested that the overall decrease in sugar content in the present study may be due to reduced photosynthesis or additional consumption of these metabolites for defense-related activities.

Virus-infected leaves (0.9%) show around 20-fold decrease in total fatty acid component as compared to healthy leaves (18.21%). In plant cells, fatty acids and lipids are essential components as they provide structural integrity and fulfil the energy demand of cells for the smooth conduction of

various metabolic processes and as signal transduction mediators. A fatty acid, n-hexadecenoic acid (Palmitic acid) which is reported in the highest amount (6.91%) in healthy leaves, found highly suitable for the integrated control of phytopathogens (Liu et al., 2008), works as a central molecule in lipid biosynthesis, facilitates transport of lipids across membranes and also have important role in biotic and abiotic stress responses (Lim *et al*., 2017). It is suggested that a drastic decrease in important fatty acids after GBNV infection may alter structural integrity and overall failure of key metabolic processes resulting in disease development. Fatty acid esters (11.32%) and phenol (0.43%) were detected only in HL not in VIL. It is supported by another study in which fatty acid methyl esters and phenolic compounds were found downregulated in squash due to cucumber mosaic virus infection (Abdelkhalek *et al*., 2022). VIL samples showed a 47-fold decrease in ketones as compared to HL. Antiviral activity of some ketones reported elsewhere (Sinha *et al*., 2019). Possibly this reduction in important metabolites shows the failure of defense mechanism of plants and results in the success of virus spread. A trace amount of niacin (vitamin) was detected in HL samples while not detected in VIL. Niacin is known as a strong antioxidant and has an impact on enzymes related to cellular energy production (Berglund *et al*., 2017). Some other metabolites such as cyclohexane derivatives, and esterified acids were also detected in VIL and few others were reported in HL but the role of these metabolites in defense against viral pathogens has not been reported to the best of our knowledge.

Figure 1 : RT- PCR detection of Groundnut Bud Necrosis Virus by amplification of nucleocapsid protein gene of 831 base pair (Cowpea samples 1 to 6, M- 1KB marker along with negative and positive control)

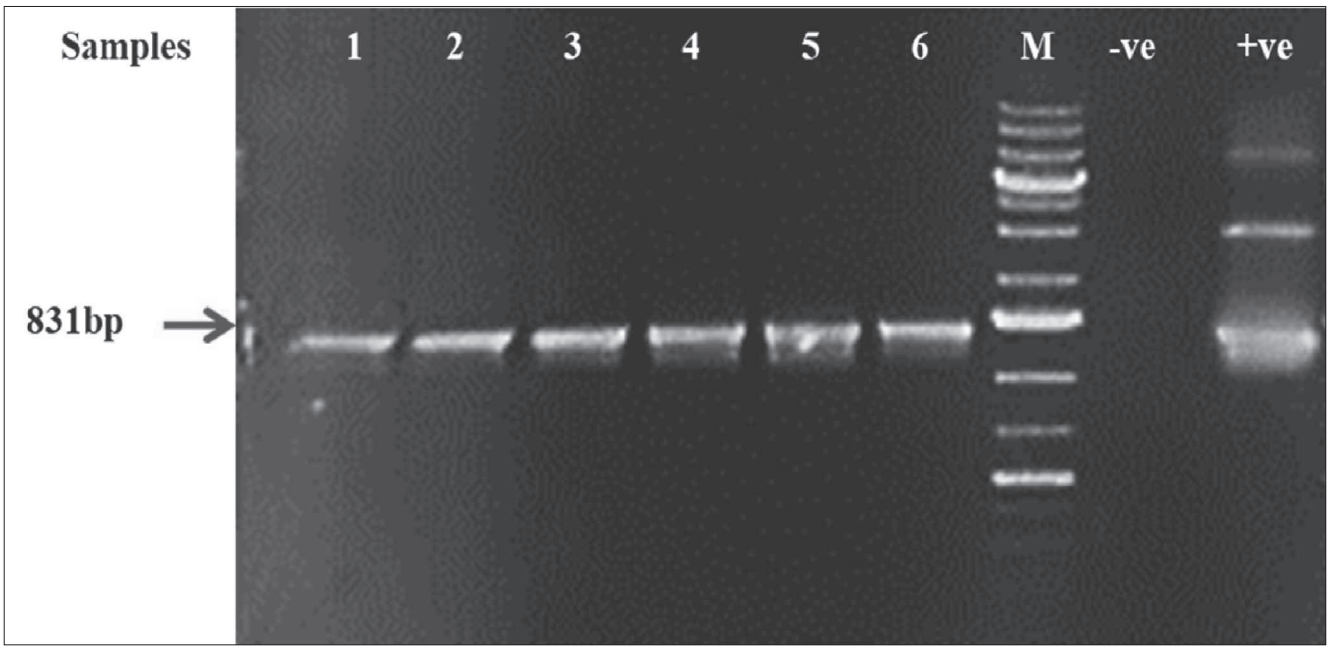

Figure 2 : GC-MS chromatograms of methanolic extract of (a) healthy (HL) and (b) Virus infected cowpea leaves (VIL)

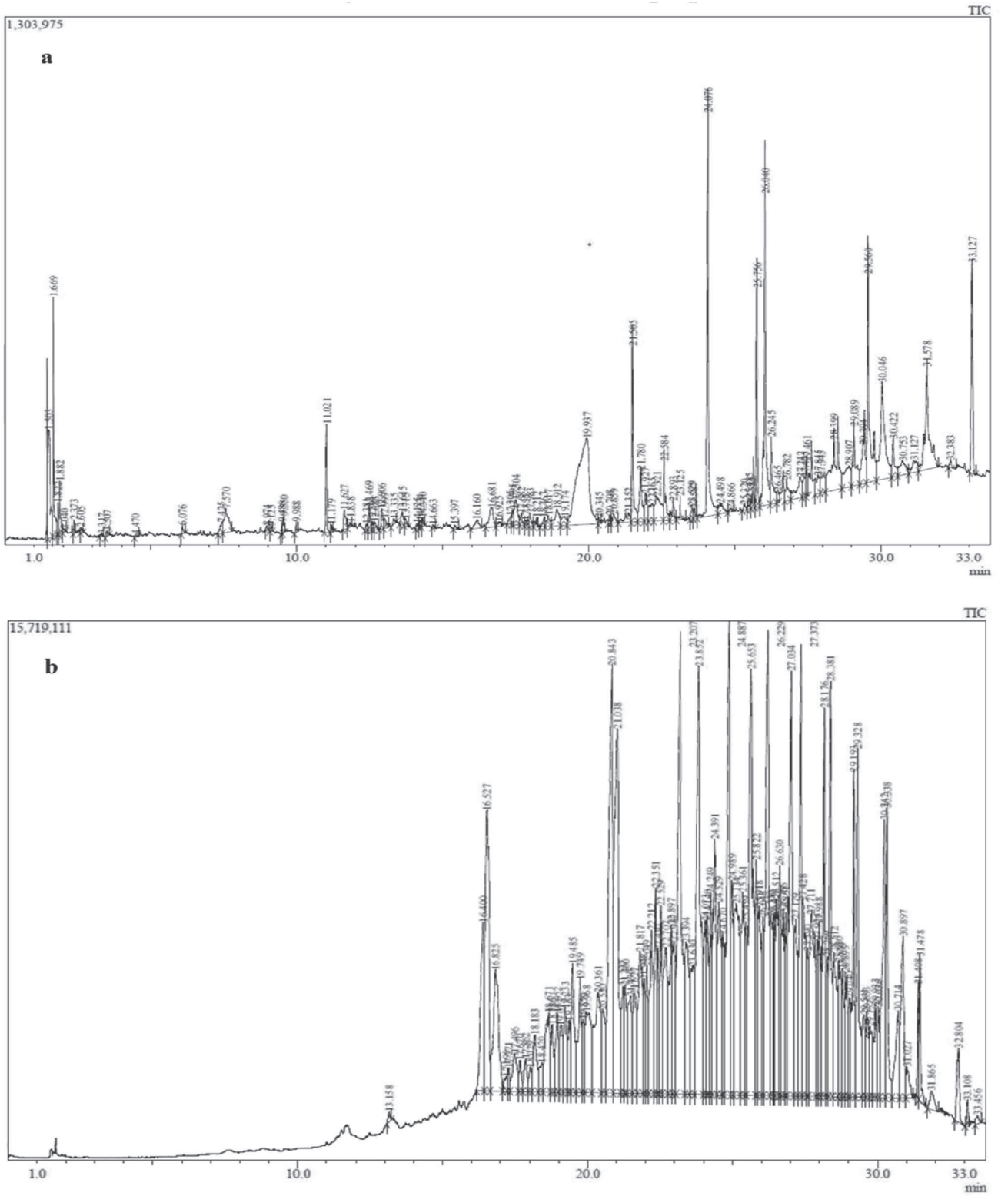

Table 1 : Metabolites detected in GC-MS analysis of healthy cowpea leaves (HL)

S. No.	Class of Metabolite	Name of Metabolite	Retention Time	Area %	Molecular Formula	Molecular Weight (g/mol)
1	Alcohol	Isopropyl Alcohol	1.669	1.99	C3H8O	60.1
		Tetrahydro-3-furanmethanol	13.715	0.2	C5H10O2	102.13
		3-Heptanol	14.125	0.09	C7H16O	116.2
		n-Tridecan-1-ol	20.345	0.11	C13H28O	200.36
		Z,E-3,13-Octadecadien-1-ol	22.893	0.47	C18H34O	266.5
2	Aldehyde	Propanal, 2-methyl-	1.822	0.4	C4H8O	72.11
		Butanal, 2-methyl-	2.373	0.11	C5H10O	86.13
3	Alkane	3-Ethyl-2,6,10-trimethylundecane	16.925	0.18	C15H32	212.41
		Dodecane-4,6-di methyl	17.395	0.27	C14H30	198
		Heptadecane, 8-methyl-	20.708	0.11	C18H38	254.5
		Tetratetracontane(Paraffin)	30.422	0.94	C44H90	619.2
4	Alkane derivative	1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	27.242	1.23	C16H32O3S	304.5
5	Diterpene	Neophytadiene	22.584	1.33	C20H38	278.5
6	Amide	Benzamide, 3-methyl-N-[2-(1-piperidyl)ethyl]	8.974	0.06	C15H22N2O	246.35
		Piracetam	14.224	0.09	C6H10N2O2	142.16
7	Aziridine (Heterocyclic compoud)	Aziridine, 1-ethenyl-	2.605	0.09	C4H7N	69.1
8	Benzofuran	Benzofuran, 2,3-dihydro-	12.469	0.43	C8H8O	120.15
		6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	21.352	0.57	C11H16O3	196.24
		2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4	18.212	0.19	C11H16O2	180.24
9	Carboxylic acid	Acetic acid	1.882	0.66	C2H4O2	60.05
		Butanedioic acid, monomethyl ester	9.988	0.13	C5H8O4	132.11
		Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	14.663	0.06	C5H8O5	148.11
		L-Proline, 1-acetyl- (Imino acid)	17.983	0.31	C7H11NO3	157.17
10	Carbonic acid ester	Carbonic acid, octadecyl prop-1-en-2-yl ester	26.465	0.25	C22H42O3	354.6
11	Cyclic lactone (Furan)	Butyrolactone	6.076	0.14	C4H6O2	86.09
12	Diazines	Pyrazine, methyl-	4.47	0.06	C5H6N2	94.11
13	Dioxolane (organic heterocyclic compound)	1,3-Dioxolane, 2,4,5-trimethyl-	16.16	0.39	C6H12O2	116.16
14	Diterpene alcohol	Phytol	23.125	2.82	C20H40O	296.5
15	Ester (Palmitic ester derivative)	Dimethylaminoethyl palmitate	27.461	0.62	C20H41NO2	327.5
16	Fatty acid	Dodecanoic acid	18.463	0.2	C12H24O2	200.32
		Tetradecanoic acid	21.505	2.91	C14H28O2	228.37
		n-Hexadecanoic acid	24.076	6.91	C16H32O2	256.42
		9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (FA Omega3)	26.04	6.73	C18H30O2	278.4
		Octadecanoic acid	26.245	1.46	C18H36O2	284.5
17	Fatty acid ester	Heptanoic acid, 3-methylbutyl ester	20.859	0.32	C12H24O2	200.32
		7-Hexadecenoic acid, methyl ester, (Z)-	23.587	0.44	C17H32O2	268.4
		Methyl (Z)-5,11,14,17-eicosatetraenoate	31.578	4.73	C21H34O2	318.5
		Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	29.56	5.83	C19H38O4	330.5
18	Furanone	4-Dimethylamino-2(5H)-furanone	18.617	0.38	C6H9NO2	127.14
19	Glycerol (monoacyl glycerol)	1,2,3-Propanetriol, 1-acetate	12.906	0.41	C5H10O4	134.13
20	Glycoside	beta.-D-Glucopyranoside, methyl	18.912	0.87	C7H14O6	194.18
21	Halogenated hydrocarbons	Ethane, 1-chloro-1-fluoro-	1.503	3.21	C2H4ClF	82.5
		Trichloromethane	2.04	0.09	CHCl3	119.37
22	Ketoacid	2-Keto-3-methylvaleric acid tbdms	13.335	0.11	C12H24O3Si	244.40
23	Ketone	2-Propanone, 1-hydroxy-	3.507	0.1	C3H6O2	74.08
		2-Propanone, 1,1-diethoxy-	7.57	1.21	C7H14O3	146.18
		Ethanone, 1-(1,2,2,3-tetramethylcyclopentyl)	9.125	0.08	C11H20O	168.28
		2-Hexanone, 3-methyl-4-methylene-	9.55	0.24	C8H14O	126.2
		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6	11.021	1.28	C6H8O4	144.12
		2(3H)-Furanone, dihydro-4-hydroxy-	11.179	0.14	C4H6O3	102.09
		4-Hexen-3-one, 4,5-dimethyl-	12.591	0.15	C8H14O	126.2
		2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one	17.835	0.1	C8H13NO2	155.19
		4,4,8-Trimethyl-non-7-en-2-one	21.927	0.96	C12H22O	182.3
24	Organofluorine	Heptadecyl heptafluorobutyrate	25.485	0.21	C21H35F7O2	452.5

S. No.	Class of Metabolite	Name of Metabolite	Retention Time	Area %	Molecular Formula	Molecular Weight (g/mol)
25	Phenol	2-Methoxy-4-vinylphenol	14.31	0.16	C9H10O2	150.17
		2,4-Di-tert-butylphenol	17.682	0.27	C14H22O	206.32
26	Pyrrolidine derivative	2-Pyrrolidinone	9.486	0.14	C4H7NO	85.1
		(2-Oxo-1-pyrrolidinyl)acetic acid	24.866	0.09	C6H9NO3	143.14
		L-Proline, 5-oxo-, methyl ester	15.397	0.1	C6H9NO3	143.14
27	Phytosterols	gamma.-Sitosterol	30.046	5.26	C29H50O	414.7
28	Diterpinoid	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	28.399	4.24	C22H42O2	338.6
29	Sugar	Sucrose	16.681	0.98	C12H22O11	342.3
		beta.-D-Glucopyranose, 1,6-anhydro-	17.504	0.92	C6H12O6	180.16
		4-O-Methylmannose	19.937	11.96	C7H14O6	194.18
30	Steroid	beta.-Sitosterol acetate	30.753	1.03	C31H52O2	456.7
31	Triterpenes	Squalene	33.127	3.49	C30H50	410.7
32	Vaccenoate	2-(Dimethylamino)ethyl vaccenoate	29.089	1.9	C22H43NO2	353.58
33	Valerolactone derivative	2H-Pyran, 2-(4-chlorobutoxy)tetrahydro-	11.838	0.1	C9H17ClO2	192.68
34	Vitamin (Group B complex)	Niacin	12.686	0.21	C6H5NO2	123.11

Table 2: Metabolites detected in GC-MS analysis of virus infected cowpea leaves (VIL)

S. No.	Class of Metabolite	Name of Metabolite	Retention Time	Area%	Molecular Formula	Molecular Weight
1	Alcohol	1-Hexacosanol	30.897	1.11	C26H54O	382.7
		dl-Chimyl alcohol	31.865	0.16	C19H40O3	316.5
2	Alkane	Heneicosane	16.4	1.08	C21H44	296.6
		Octadecane	16.527	2.13	C18H38	254.5
		Dodecane-4,6-di methyl	17.19	0.09	C14H30	198
		2-Methylhexacosane	17.862	0.2	C27H56	380.7
		Pentadecane, 8-hexyl-	18.183	0.42	C21H44	296.6
		3-Ethyl-2,6,10-trimethylundecane	18.671	0.78	C16H34	226.44
		Pentadecane, 6-methyl-	18.786	0.31	C16H34	226.44
		Decane, 3,3,6-trimethyl-	19.233	0.54	C13H28	184.36
		Heptadecane, 2-methyl-	19.485	0.83	C18H38	254.5
		Octadecane, 3-methyl-	19.749	0.77	C19H40	268.5
		Eicosane	21.038	2.97	C20H42	282.5
		Pentacosane	21.49	0.95	C25H52	352.7
		2-Methylhexacosane	21.627	0.73	C27H56	380.7
		Pentadecane, 6-methyl-	21.948	0.48	C16H34	226.44
		Heneicosane, 5-methyl-	22.049	0.59	C22H46	310.6
		Docosane	22.212	1.12	C22H46	310.6
		Octacosane	22.351	0.81	C28H58	394.8
		Eicosane, 2-methyl-	22.897	1.07	C21H44	296.6
		3,3-Dimethylnonadecane	24.14	0.71	C21H44	296.6
		Hexatriacontane	24.391	1.78	C36H74	507
		Tetratetracontane	24.989	1.04	C44H90	619.2
		Pentadecane, 6-methyl-	25.459	0.67	C16H34	226.44
		Octacosane, 2-methyl-	25.822	0.83	C29H60	408.8
		Pentadecane, 2,6,10,14-tetramethyl-	26.048	0.77	C19H40	268.5
		5-Methylnonacosane	26.33	0.97	C30H62	422.8
		2-Methyltetracosane	26.766	1.01	C25H52	352.7
		Tetracosane	27.034	2.71	C24H50	338.7
		Dotriacontane	27.428	1.31	C32H66	450.9
		Docosane, 2,4-dimethyl-	27.59	0.43	C24H50	338.7
		2-Methylpentacosane	28.895	0.7	C26H54	366.7
		3-Methyltetracosane	29.04	0.37	C25H52	352.7

S. No.	Class of Metabolite	Name of Metabolite	Retention Time	Area%	Molecular Formula	Molecular Weight
		Tetratriacontane	29.193	1.38	C34H70	478.9
		Triacontane	29.328	1.45	C30H62	422.8
		Tetracontane	29.633	0.48	C40H82	563.1
		2-Methylhexacosane	30.036	0.43	C27H56	380.7
3	Alkene	Nonacos-1-ene	18.42	0.22	C29H58	406.8
4	Benzene derivative	Benzene, (2,3-dimethyldecyl)-	19.968	0.96	C18H38	246.4
5	Citronellyl linoleate	(9Z,12Z)-3,7-Dimethyloct-6-en-1-yl octadeca-	18.029	0.13	C28H50O2	418.7
6	Cyclohexane derivative	1,1'-Bicyclohexyl	19.363	0.3	C12H22	166.3
		Heneicosylcyclohexane	22.43	0.53	C27H54	378.7
		Cyclohexane, nonadecyl-	22.982	0.78	C25H50	350.7
		n-Heptadecylcyclohexane	27.179	1.14	C23H46	322.6
7	Diterpene	Hexadecane, 2,6,10,14-tetramethyl- (Phytane)	16.825	1.37	C20H42	282.5
8	Esterified acid	Sulfurous acid, hexyl pentadecyl ester	18.952	0.43	C21H44O3S	376.6
		Oxalic acid, cyclohexylmethyl tridecyl ester	21.237	0.63	C22H40O4	368.5
		Sulfurous acid, cyclohexylmethyl hexadecyl ester	21.3	0.4	C23H46O3S	402.7
		Sulfurous acid, di(cyclohexylmethyl) ester	23.63	1.08	C14H26O3S	274.4
		Sulfurous acid, butyl heptadecyl ester	24.67	0.55	C21H44O3S	376.6
9	Ether	Hexadecyl nonyl ether	17.496	0.37	C25H52O	368.7
		Nonyl tetracosyl ether	27.924	0.51	C33H68O	480.9
10	Fatty acid	n-Hexadecanoic acid	24.073	0.9	C16H32O2	256.42
11	Fatty alcohol	1-Heptacosanol	20.53	0.62	C27H56O	396.7
12	Fatty acid (glyceride)	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	29.541	0.51	C19H38O4	330.5
13	Halogenated hydrocarbon	Hexadecane, 1-iodo-	13.158	0.04	C16H33I	352.34
		Tetrapentacontane, 1,54-dibromo-	17.67	0.14	C54H108Br2	917.2
		Hexacosane, 1-iodo-	24.249	0.82	C26H53I	492.6
		Triacontane, 1-iodo-	25.134	2.09	C30H61I	548.7
		Eicosane, 1-iodo-	26.851	0.67	C20H41I	408.4
14	Ketone	3-Dodecylcyclohexanone	17.271	0.09	C18H34O	266.5
15	Organofluorine	Triacontyl heptafluorobutyrate	30.714	0.7	C34H61F7O2	634.8
16	Triterpene	Squalene	33.108	0.07	C30H50	410.7
17	Triterpinoid	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	33.456	0.05	C30H50O	426.7

Table 3 : Comparative list of area percent of different metabolite classes with fold change in area percent of common metabolites in HL and VIL samples

S.No.	Class of Metabolite	Peak area percent		Fold change area percent
		HL	VIL	
1	**Alcohol**	**2.86**	**1.27**	**↓ 2.25**
2	Aldehyde	0.51	_	
3	**Alkane**	**1.5**	**32.91**	**↑ 21.94**
4	Alkane derivative	1.23	_	
5	Alkene	_	0.22	
6	Amide	0.15	_	
7	Aziridine	0.09	_	
8	Benzene derivative	_	0.96	
9	Benzofuran	1.19	_	
10	Carboxylic acid	1.16	_	

S.No.	Class of Metabolite	Peak area percent		Fold change area percent
		HL	VIL	
11	Carbonic acid ester	0.25	_	
12	Citronellyl linoleate	_	0.13	
13	Cyclic lactone	0.14	_	
14	Cyclohexane derivative	_	2.75	
15	Diazines	0.06	_	
16	Dioxolane	0.39	_	
17	**Diterpenes**	**1.33**	**1.37**	**↑ 1.03**
18	Diterpene alcohol	2.82	–	
19	Diterpenoid	4.24	–	
20	Ester	0.62	–	
21	Esterified acid	–	3.09	
22	Ether	–	0.88	
23	**Fatty acid**	**18.21**	**0.9**	**↓ 20.23**
24	**Fatty acid ester**	**11.32**	**0.51**	**↓ 22.19**
25	Fatty alcohol	–	0.62	
26	Furanone	0.38	–	
27	Glycerol	0.41	–	
28	Glycoside	0.87	–	
29	**Halogenated hydrocarbon**	**3.3**	**3.76**	**↑ 1.14**
30	Ketoacid	0.11	_	
31	**Ketone**	**4.26**	**0.09**	**↓ 47.33**
32	**Organofluorine**	**0.21**	**0.7**	**↑ 3.33**
33	Phenol	0.43	–	
34	Pyrrolidine derivative	0.33	–	
35	Phytosterols	5.26	–	
36	Sugar	13.86	–	
37	Steroid	1.03	–	
38	**Triterpenes**	**3.49**	**0.07**	**↓ 49.86**
39	Triterpinoid	_	0.05	
40	Vaccenoate	1.9	–	
41	Valerolactone derivative	0.1	–	
42	Vitamin (Niacin)	0.21	–	

CONCLUSION

Our results showed that the volatile metabolites profile of GBNV infected cowpea leaves was mostly different from healthy leaves. Some important metabolite classes such as sugars, fatty acids, fatty acid esters, triterpenes, triterpenoids, ketones, alcohols and phytosterols were reduced significantly or even not detected in leaves of cowpea after GBNV infection while some other metabolite classes such as alkanes, halogenated hydrocarbons, cyclohexane derivatives, esterified acids and diterpenes

were overexpressed after GBNV infection. Overall, this analysis suggests that GBNV causes a profound effect on host volatile phytochemical composition and alters it significantly for successful disease development.

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