IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11, Iss 11 NOV 2022

Identification of primary metabolite of tomato grown in different parts of Meghalaya, India

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ABSTRACT

The state of Meghalaya is well-known for both its horticulture crop types and tourism. One of the major crops that is grown all year round in Meghalaya is the tomato. Metabolites are used to assess the quality of tomatoes from various regions in Meghalaya. LCMS spectroscopy was used to discover primary metabolites in eleven tomato genotypes from various regions of Meghalaya. Primary health-promoting metabolites were present in all genotypes that were analyzed; however, the round-shaped tomato from Baghmara, which measured 4.9 cm in diameter, and the egg-shaped tomato from Resubelpara, which had an average diameter of 4.4 cm, were found to be a rich source of these metabolites, whereas the genotypes collected from Tura, Jowai, Nongpoh, and Mawkyrwat had the fewest number of primary metabolites. Given the reported differences in metabolite composition, it may be possible to use breeding techniques and genotypic selection to improve particular health-promoting traits in tomatoes.

KEYWORDS: Metabolites, LCMS, primary metabolites, secondary metabolites.

One of the most significant horticultural crops in the world, tomatoes (*Solanum lycopersicum L.*) are prized for their large nutritional value and economic importance (Bergougnoux, 2014). Tomato fruits are abundant in primary metabolites, which are important for flavour, nutritional quality, and fruit development. These include sugars (glucose, fructose), organic acids (citric acid, malic acid), and amino acids (glutamic acid, aspartic acid), in addition to vitamins and minerals (Baldina *et a*l., 2016). Genotype, environmental conditions, and their interactions all affect how much primary metabolite accumulates in tomatoes. Acid biosynthesis, amino acid buildup, and glucose metabolism are all impacted by agroclimatic factors such temperature, light intensity, humidity, soil nutrients, and altitude (Tieman *et al*., 2017). Tomatoes are used in processed foods including soups, sauces, and juices, in addition to being eaten as fresh fruits (Li et al., 2018b). Consumers have learnt more about foods' health advantages and how they may help avoid a variety of chronic illnesses and dysfunctions during the past ten years (Pem and Jeewon, 2015). Tomato nutritional value was predicted by health-promoting substances such vitamins, carotenoids, and phenolic compounds. (Li *et al*., 2018b; Martí *et al*., 2016; and Raiola *et al*., 2014). According to Raiola *et al*. (2014), the bioactive chemicals listed exhibit a wide range of physiological activities, including anti-inflammatory, antibacterial, anti-allergenic, vasodilatory, antithrombotic, cardioprotective, and antioxidant actions. In addition to nutritional

value, carotenoids and polyphenolic compounds also improve the sensory qualities, including taste, aroma, and texture of tomatoes (Tohge *et al*., 2015; Martí *et al*., 2016). A large number of metabolites, such as sucrose, hexoses, citrate, malate, and ascorbic acid, are also present in tomatoes. (Li *et al*, 2018b). For these kinds of studies, Meghalaya, in northeastern India, provides a special natural laboratory. At elevations between 50 to more than 1,900 meters above sea level, it includes a variety of microclimates, as well as mid-hill, subtropical, and temperate highlands. These diverse circumstances affect the biochemical makeup, yield, and phenology of tomatoes. There aren't many systematic researches on the primary metabolite diversity of tomato genotypes across the state's agroecological zones, despite the fact that the state is recognised for having ideal growing conditions for tomatoes all year round. Thus, identifying and contrasting the major metabolite profiles of tomato genotypes gathered from various regions of Meghalaya is the goal of the current study. By linking biochemical variation to environmental and genetic factors, this research seeks to provide valuable insights for tomato improvement programmes and the promotion of nutritionally superior cultivars suited to regional conditions.

MATERIALS AND METHODS:

Eleven tomato genotypes collected from eleven distinct locations are provided in Table 1

Table 1. Tomato Genotypes collection from different locations and their physical characteristics.

Genotypes collection from location (Villages)	District	Shape	Average Diameter (cm)	Latitude	Longitude
Ampati	South west Garo Hills	Egg like	5.0±0.2	25˚27’49’’N	89˚55’46’’E
Dalu	West Garo Hills	Round	5.1±0.2	25˚12’56’’N	90˚13’39’’E
Resubelpara	North Garo Hills	Egg like	4.4 ±0.2	25˚90’41’’N	90˚60’62’’E
Tura	West Garo Hills	Cherry like	1.9 ±0.1	25˚30’36’’N	90˚12’59’’E
Baghmara	South Garo Hills	Round	4.9±0.2	25˚19’35’’N	90˚63’46’’E
Williamnagar	East Garo Hills	Cherry like	2.0±0.1	25˚53’14’’N	90˚59’20’’E
Shillong	East Khasi Hills	Egg like	4.2±0.2	25˚57’88’’N	91˚89’33’’E
Jowai	West Jaintia Hills	Round	5.1±0.2	25˚30’00’’N	92˚15’00’’E
Nongpoh	Ri-Bhoi	Egg like	3.8±0.1	25˚86’99’’N	91˚83’37’’E
Mawkyrwat	South West Khasi Hills	Round	4.6 ±0.2	25˚36’25’’N	91˚45’56’’E
Khliehriat	East Jaintia Hills	Egg like	4.3 ±0.2	25˚35’67’’N	92˚36’41’’E

Sample preparation

The fruit pericarp of tomato fruit was dried at room temperature for seven days. These were ground to obtain the powder form. Added 10 mL methanol to 1 mg of powdered sample, kept it for 4 hours, and then the samples were centrifuged for 10 min at 5000 rpm. Centrifuged samples were performed for LCMS analysis.

Liquid chromatography mass spectrometry analysis

The extraction was analysed using liquid chromatography mass spectrophotometry (LCMS) on a single quadruple LCMS system that was fitted with a capillary column. Each sample extract was centrifuged and separately diluted. The ion source was set to 230°C with the start time of (solvent delay) = 2 min (solvent cutting). The temperature was kept at 40°C for 4 min and gradually increased to 280°C at a rate of 20°C/min.

The overall run time was 30 min. The temperature at the LC and the trans-line sections was greater (280°C) than that at the MS section (250°C) to ensure that all ions have fully shifted to the MS section. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The electron impact ionisation voltage was 70 eV. The compounds were analysed for constituent identification using total ion count (TIC) after being compared to a database of known components available in the computer library attached to the LCMS instrument. The instrument was injected with 10 µL of the sample in split mode. An Rtx5MS-30m column with 0.25 mm ID and 0.25 µm df was used. Using a scanning range of 40 to 850 m/z, mass spectra were obtained at 2 scan sec^{-1}.. Each component's peak regions were quantified,, and normalisation was conducted using an internal standard. In the chromatograms the x-axis indicates the retention period, while the y-axis represents the absorbance units AU (a signal corresponding to the detector's response) at 210 nm. The records were made in retention time of the first 10 min and absorbance units up to a maximum of 0.070. The chromatograms displayed the components based on their retention period and mass-to-charge ratio, represented by their relative abundance.

Results

Primary Metabolites

Numerous metabolites that are abundant in tomatoes are essential to their nutritional value and metabolic makeup. Eleven different tomato genotypes have had a wide variety of metabolites discovered by liquid chromatography-mass spectrometry (LC-MS) study. The primary metabolites identified in these tomato genotypes include: Lignoceric acid, cis-Cinnamic acid, Stearic acid, Isonicotinic acid, cis-Aconitic acid, Niflumic acid, 3-Phenylpropionic acid, Fenofibric acid, 1-Hexanoic acid, Cer[NS] d46:2, Phosphatidylcholine lyso 16:1, Phosphatidylcholine lyso, Phosphatidylserine, Phosphatidylcholine 20:1-22:6, Biotin, Pyridoxine, 2-Amino-1,3-dihydroxyoctadecane, L-Arginine monohydrochloride, 2-Aminoadipic acid, gamma-L-Glutamyl-L-cysteine, N-Glycolylneuraminic acid, N-Methylglutamic acid, Hippuric acid, Lauramidopropyl betaine, Blasticidin S, and 5-oxo-L-prolyl-glycine. These metabolites have been further categorised into five main groups(amino alcohols, lipids, vitamins, carboxylic acids, and amino acids) according to their chemical structures and functional groups in order to help us better understand their biochemical roles (Nunes-Nesi et al., 2019).

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 Journal UGC CARE Listed (Group-I) Volume 11, Iss 11 NOV 2022

Table 10: List of Amino acids detected in the samples is arranged in increasing order of retention times in chromatograms.

SL.No	RT(min)	m/z value	Genotype No.	Name of the compound	Mol. Formula and Mol.mass (g/mol)
1	0.750	125.56	11	N-Glycolylneuraminic acid	$C_{11}H_{19}NO_{10}$, 325.27
2	0.783	158.76	7	5-oxo-L-prolyl-glycine.	$C_7H_{10}N_2O_4$, 186.17
3	1.253	313.6	7	Blasticidin S	$C_{17}H_{26}N_8O_5$, 422.0
4	1.616,	142.57	10	N-Methylglutamic acid	$C_6H_{11}NO_4$, 161.16
5	1.619	241.39	8	Lauramidopropyl betaine	$C_{17}H_{36}N_4O_3$, 344.49
6	1.938	179.5	11	Hippuric acid	$C_9H_9NO_3$, 179.17
7	8.332	122.92	5	gamma-L-Glutamyl-L-cysteine	$C_8H_{14}N_2O_5S$, 250.27
8	8.661	121.97	7	2-Aminoadipic acid	$C_6H_{11}NO_4$, 161.17
9	9.144	174.07	5	L-Arginine monohydrochloride	$C_6H_{15}ClN_4O_2$, 210.66

Table-11: List of Carboxylic acids detected in the samples is arranged in increasing order of retention times in chromatograms.

SL.No	**RT(min)**	**m/z value**	**Genotype No.**	**Name of the compound**	**Mol. Formula and Mol. mass (g/mol)**
1	0.435	106.2	3	Lignoceric Acid	$C_{24}H_{48}O_2$, 368.63
2	0.750	284.5	8	Stearic acid	$C_{18}H_{36}O_2$, 284.48
3	0.750	125.6	11	Isonicotinic acid	$C_6H_5NO_2$, 123.11
4	1.247	129.7	3	cis-Aconitic acid	$C_6H_6O_6$, 174.11
5	1.249	214.9	1	Niflumic acid	$C_{13}H_9F_3N_2O_2$, 282.21
6	2.875	166.6	9	Quinolinic acid	$C_7H_5NO_4$,167.12
7	3.844	233.8	1	Fenofibric acid	$C_{17}H_{15}ClO_4$, 318.75
8	3.915	233.8	2	Fenofibric acid	$C_{17}H_{15}ClO_4$, 318.75
9	7.786	159.76	3	3-Phenylpropionic acid	$C_9H_{10}O_2$,150.17
10	7.789	126.0	6	cis-Cinnamic acid	$C_9H_8O_2$, 148.17
11	8.660	115.9	4	1-Hexanoic acid	$C_6H_{12}O_2$,116.16

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Table-12: List of Lipids detected in the samples is arranged in increasing order of retention times in chromatograms

SL No.	RT(min)	m/z value	Sample No.	Name of the compound	Mol. Formula and Mol.mass (g/mol)
1	3.562	703.39	7	Cer[NS] d46:2	C46H89NO3 , 703.0
2	3.836	831.17	6	Phosphatidylcholine 20:1-22:6	C50H86NO8P , 860.2
3	3.850	552.0	3	Phosphatidylcholine lyso	C23H48NO7P ,481.6
4	4.068	720.95	5	Phosphatidylserine	C44H74NO10P, 808.0.
5	7.231	478.9	5	Phosphatidylcholine lyso 16:1	C24H48NO7P, 493.0

Table-13: List of Vitamins detected in the samples is arranged in increasing order of retention times in chromatograms.

Sl No.	RT(min)	m/z value	Sample No.	Name of the compound	Mol. Formula and Mol. mass (g/mol)
1	0.499	125.92	9	Biotin.	$C_{10}H_{16}N_2O_3S$, 244.31
2	8.864	122.85	2	Pyridoxine	$C_8H_{11}NO_3$, 169.18

Table-14: List of Amino alcohol detected in the samples is arranged in increasing order of retention times in chromatograms.

SL No	RT(min)	m/z value	Sample No.	Name of the compound and class	Mol. Formula and Mol.mass (g/mol)
1	3.615	302.65	2	*Sphinganine*	$C_{18}H_{39}NO_2$, 301.52

Chromatograms of samples are given in figures 5-15

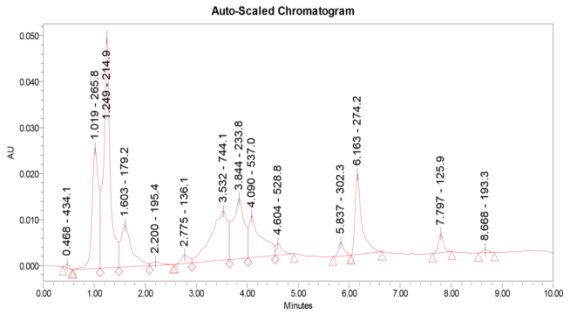

Fig 5: LCMS Chromatogram of genotype -1

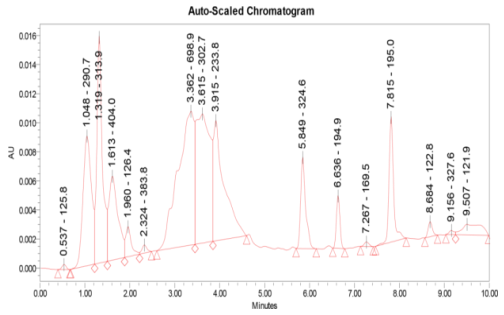

Fig 6: LCMS Chromatogram of genotype -2

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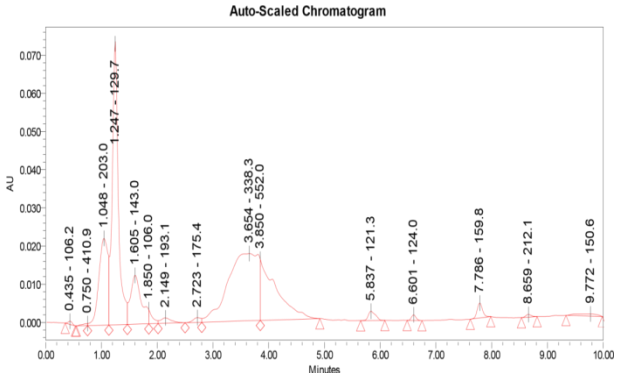

Fig 7: LCMS Chromatogram of genotype -3

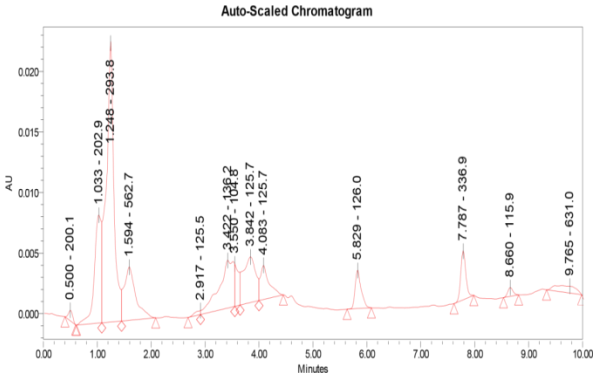

Fig 8: LCMS Chromatogram of genotype -4

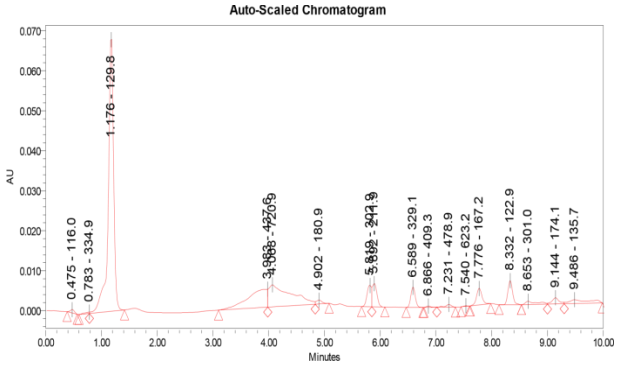

Fig 9: LCMS Chromatogram of genotype -5

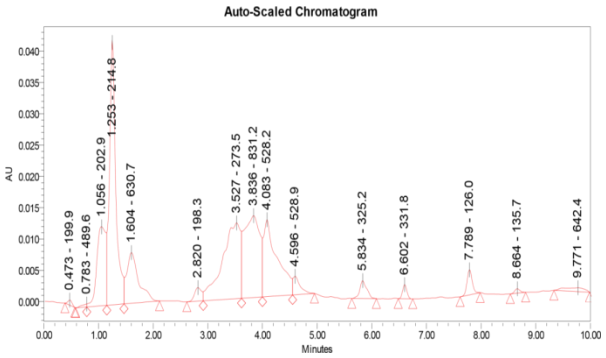

Fig 10: LCMS Chromatogram of genotype -6

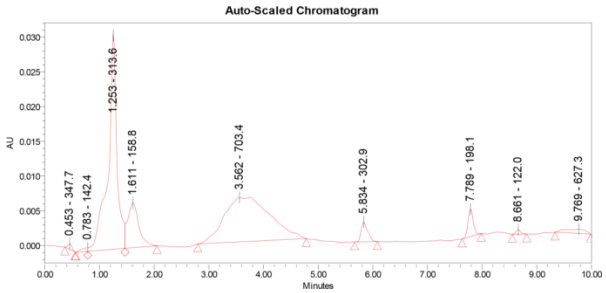

Fig 11 LCMS Chromatogram of genotype -7

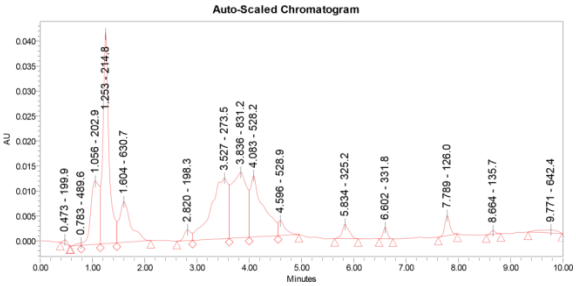

Fig 12: LCMS Chromatogram of genotype -8

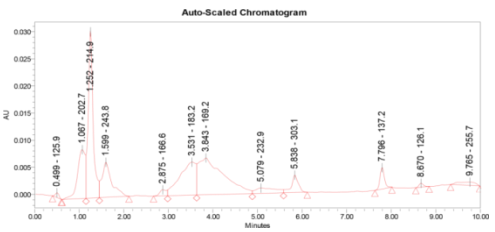

Fig 13: LCMS Chromatogram of genotype -9

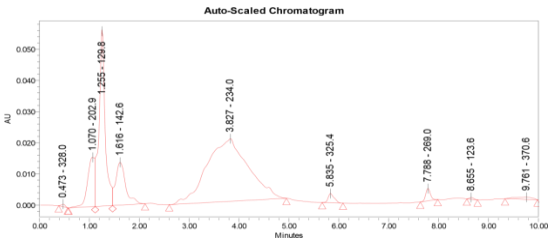

Fig 14: LCMS Chromatogram of genotype -10

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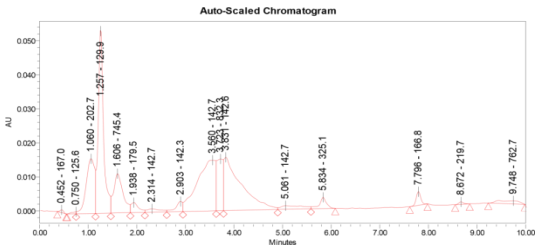

Fig 15: LCMS Chromatogram of genotype -11

Discussion

Growth, immunity, energy metabolism, and disease prevention all depend on primary metabolites. Malnutrition, reduced immunity, metabolic abnormalities, and chronic health issues can result from an imbalance or lack in these substances (Ling *et al*., 2023). The most primary metabolites were found in the genotypes of Resubelpara and Baghmara, which were followed by those from Shillong, Ampati, Dalu, Williamnagar, and Khliehriat. The fewest primary metabolites were found in the genotypes from Tura, Jowai, Nongpoh, and Mawkyrwat. The eleven genotypes can thus be arranged as follows: Resubelpara and Baghmara first, followed by Shillong, Ampati, Dalu, Williamnagar, Khliehriat, and then Tura, Jowai, Nongpoh, and Mawkyrwat, in decreasing order of the number of primary metabolites. Genotype, fruit developmental stage, and environmental variables including temperature, light, water, and soil nutrients all have a major impact on the variance of primary metabolites in tomatoes. The amounts of sugars, organic acids, and amino acids that define the quality and nutritional value of fruit are also changed by postharvest management, biotic and abiotic stressors, and other factors.

Conclusion

This study demonstrates the presence of health-promoting primary metabolites that contribute to the functional and therapeutic qualities of different tomato genotypes, highlighting their nutritional and bioactive potential. The variations in metabolite composition among tomato types highlight their distinct biochemical profiles, which can have a big impact on nutrition and human health. Primary health-promoting metabolites were present in all genotypes that were analyzed; however, the round-shaped tomato from Baghmara, which measured 4.9 cm in diameter, and the egg-shaped tomato from Resubelpara, which had an average diameter of 4.4 cm, were found to be a rich source of these metabolites, whereas the genotypes collected from Tura, Jowai, Nongpoh, and Mawkyrwat had the fewest number of primary metabolites. These results support the potential uses of several tomato genotypes in functional meals, nutraceuticals, and dietary supplements by highlighting their nutritional value and biochemical diversity. Given the reported differences in metabolite composition, it may be possible to use breeding techniques and genotypic selection to improve particular health-promoting traits in tomatoes. In order to confirm the therapeutic potential of these metabolites in human health, future research should concentrate on further biochemical characterisation, bioavailability studies, and clinical trials.

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