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Comparative GC-MS analysis of phytochemical compounds in seeds of Hebbal Avare-3 (*Lablab purpureus* var. *lignosus*) and its high-yielding M_3 generation mutants

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Abstract

Field bean (*Lablab purpureus var. lignosus*) is a multipurpose leguminous plant belonging to the Fabaceae family, extensively cultivated in tropical regions and known for its nutritional values and numerous pharmacological properties. Limited genetic variability poses significant challenges to traditional crop improvement methods. However, mutation breeding offers a promising alternative for enhancing field bean traits. This study utilizes GCMS (gas chromatography mass spectrometry) analysis to compare the phytochemical profiles of Hebbel Avare-3 (HA-3) and its high-yielding M_3 generation mutants namely, M8-18, 10 mM and 100 Gy. HA-3 exhibited the presence of 32 phytochemical compounds, while M8-18 contained 44, 10 mM revealed 32 and 100 Gy showed 36 phytochemical compounds. The mutants revealed ten abundant novel phytochemicals that were not detected in HA-3, such as 1,3-propanediol, 2-(hydroxymethyl)-2-nitro-, 9,12-octadecanoic acid, 2,3-dihydroxypropyl ester, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, stigmasterol, γ-sitosterol, 9,11-octadeca-dienoic acid, methyl ester,(E,E)-, octadecanoic acid, 2,3-dihydroxypropyl ester, γ-tocopherol, 9,12-octadecadienoic acid (Z, Z)-,2,3-dihydroxypropyl ester, octanoic acid, 2-furanylmethyl ester, which have various pharmaceutical, agrochemical and food industry applications. M8-18 is rich in 1,3-propanediol, 2-ethyl-2-(hydroxymethyl) (20.60%), 10 mM in stigmasterol (13.40%) and 100 Gy in 1,3-propanediol, 2-(hydroxymethyl)-2-nitro (19.37%). These newly identified compounds in seeds possess antifungal properties, anticancer, antibacterial and anti-inflammatory properties. The enhanced phytochemical profiles of these mutants will aid in developing a superior field bean variety with various applications in pharmaceuticals and for human consumption.

Keywords

field bean; GCMS analysis; mutation breeding; pharmacological properties; phytochemicals

Introduction

Lablab (*Lablab purpureus* L.), also known as Indian bean, sem, hyacinth bean or field bean, is one of the oldest cultivated crops. It belongs to the Fabaceae family, has a somatic chromosome number of 2n=22 and holds a distinct place among legumes for its use as a vegetable (1). The dolichos bean is also known as field bean, hyacinth bean, country bean, Indian bean, Egyptian bean, sem, wal, avare and avarai (2, 3). Field bean is extensively cultivated as a valuable grain legume in

India, Africa and various tropical and subtropical regions. It is grown throughout India and is also referred to as "poor man's bean" (4). Field bean predominantly being a self-pollinated crop, has limited variability, which poses challenges for crop improvement programs, as genetic variability is crucial for selecting the best ideotypes.

Mutation breeding is an effective method to enhance genetic diversity, particularly for economically important crop like field bean, where hybridization poses challenges. Due to the small size of flowers and low seed setting in emasculated buds, genetic variability through traditional methods has not yielded desirable results (4). In a previous study, seeds of the HA-3 variety were irradiated with gamma rays using Cesium-137 (^{137}Cs) at the National Research Centre for Banana, Trichy. The radiation doses were 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy and 350 Gy, with untreated seeds as controls. Additionally, the seeds were soaked in distilled water for 6 hours, treated with ethyl methyl sulfonate (EMS) for another 6 hours and then washed with running water for 30 minutes. EMS concentrations used were 10 mM, 20 mM, 30 mM, 40 mM, 50 mM and 60 mM, with seeds soaked in phosphate buffer serving as controls (5).

Among the various mutants produced, the ones subjected to 100 Gy, 10 mM and a viable mutant derived from 200 Gy possess the highest yield in M_3 generation. The present study focuses on the GC-MS analysis of Hebbel Avare-3 and its high-yielding M_3 generation mutants namely M8-18, 10 mM and 100 Gy. The aim of identifying and comparing the phytochemical profiles of these mutant varieties is to understand their potential health benefits and agricultural implications, ultimately leading to the development of a superior field bean variety.

Materials and Methods

Sample collection

Dry seeds of HA-3 and its derived high-yielding M_3 mutants (M8-18, 10 mM and 100 Gy) were collected from the Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Preparation and extraction of sample

Five grams of dried seeds from each variety were powdered and extracted with 150 mL of methanol in a Soxhlet apparatus at 64.8°C for 8 hours. The resulting methanolic extract was filtered using Whatman No.1 filter paper. The filtrate was allowed to dry at room temperature. The resulting dried powder was then collected in sterile, screw-capped bottles, properly labeled and stored for subsequent experiments (6).

GC-MS analysis

Phytochemicals analysis was carried out using an Agilent GC 8890/ MS5977C/ Autosampler 7693A, equipped with DB-5ms column (30 m length / 0.25 mm internal diameter / 0.25 µm film thickness). Helium gas (99.999% purity) was used as carrier gas at a flow rate 1 mL/min. The oven temperature was programmed as follows: 50°C for 1 min, then increased a rate of 10°C/min to 300°C, where it was for 1 min.

Statistical analysis

Mass Hunter software was used for data acquisition and interpretation of chromatograms and mass spectra. The National Institute of Standard and Technology (NIST) 20 library was used to obtain the names, molecular weight and identification of compounds.

Results and Discussion

The chemical composition of HA-3 seeds and their mutants was successfully identified using GC-MS, revealing differences in the phytochemical profiles between HA-3 and its mutants, as well as among the various mutants themselves. HA-3 contained 32 phytochemical compounds, while M8-18 exhibited 44 phytochemical compounds, 10 mM revealed the presence of 32 phytochemical compounds and 100 Gy contained 36 phytochemical compounds, along with their retention time and percentage areas. Among the various identified phytochemicals, the top ten compounds, with the highest peak area percentage in HA-3 and its mutants are displayed in Table 1, while the most abundant phytochemical with their function is tabulated in Table 2.

The current study of HA-3 and its mutants has revealed significant changes in their metabolic profiles, leading to the production of new phytochemicals with diverse functions. The three most abundant phytochemicals present in HA-3 are *n*-hexadecanoic acid (15.11 %), 9,12-octadeca-dienoic acid (Z, Z)- (13.58%) and 9,12-octadeca-dienoic acid (Z, Z)-, methyl ester (11.29%). Around ten new abundant phytochemicals with various functions were found in mutants of HA-3 such as 1,3-propanediol, 2-(hydroxymethyl)-2-nitro-, 9,12-Octadeca-noic acid, 2,3-dihydroxypropyl ester, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, stigmasterol, γ- sitosterol, 9,11-octadecadienoic acid, methyl ester, (E,E)-, octadecanoic acid, 2,3 -dihydro-xypropyl ester, γ-tocophe-rol, 9,12-octadecadienoic acid (Z,Z)-,2,3-dihydroxypropyl ester and octanoic acid, 2-furanylmethyl ester. A heat map is provided, where each row represents one of the variety/mutant and each column represents a different phytochemical (Fig. 1). The color of each cell indicates the concentration of that specific phytochemical in the respective variety/mutant. The data are displayed as a color gradient, typically ranging from cool to warm colors, indicating low to high concentrations.

Among the mutants, M8-18 shows the highest amounts of 1,3-propanediol, 2-ethyl-2-(hydroxymethyl) (20.60%) and 9,12 -octadecadienoic acid (Z, Z)-2,3-dihydro-xypropyl ester (9.51%), 10 mM has the highest amounts of stigmasterol (13.40%), 9,11-octadecadienoic acid methyl ester (E, E) (9.99%) and γ-sitosterol (7.48%) and 100 Gy contains the highest amounts of 1,3-propanediol, 2-(hydroxymethyl)-2-nitro (19.37%), 9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester (11.99%) and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (8.53%). The amount of 1,3-propanediol, 2-ethyl-2-(hydroxymethyl), which has antibacterial and anti-inflammatory properties, increased almost two folds in M8-18 (20.60%) compared to HA-3 (11.44%). The Venn diagram illustrates the distribution of compounds identified in the GC-MS analysis across HA-3, M8-18, 10 mM and 100 Gy (Fig. 2). It displays the number of unique and shared compounds among HA-3 and its mutants, with counts and percentages shown in each section. These findings suggest that the mutants of HA-3 have undergone

Table 1. Top 10 phytochemicals identified from HA-3 and its mutants

Variety/ Mutant	RT time	Compound	Molecular formula	Molecular wt. (g/mol)	Area %
HA-3	15.1778	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	$C_6H_{14}O_3$	134.17	11.44
	18.8484	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	15.11
	20.2868	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.5	11.29
	20.8366	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	13.58
	18.3131	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	5.76
	26.0003	Squalene	$C_{30}H_{50}$	410.7	5.41
	24.4963	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330.5	4.27
	20.7638	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	2.94
	20.7201	Cis-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.5	2.88
	20.1921	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.5	2.46
M8-18	15.2507	1,3-Propanediol, 2-ethyl-2 (hydroxymethyl)-	$C_6H_{14}O_3$	134.17	20.60
	26.1387	9,12-Octadecadienoic acid (Z,Z)-,2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354.5	9.51
	22.2569	Octanoic acid, 2-furanylmethyl ester	$C_{13}H_{20}O_3$	224.3	7.00
	24.4782	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5	6.47
	20.2759	9,11-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.5	6.16
	20.8185	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	5.69
	25.9858	Squalene	$C_{30}H_{50}$	410.7	4.43
10 mM	18.8230	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	3.90
	18.3023	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	3.49
	26.0222	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.6	3.18
	25.4286	Stigmasterol	$C_{29}H_{48}O$	412.7	13.40
	20.2686	9,11-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.5	9.99
	26.8123	γ-Sitosterol	$C_{29}H_{50}O$	414.7	7.48
	24.4745	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330.5	7.24
	15.1669	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	$C_6H_{14}O_3$	134.17	7.16
	18.2985	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	5.75
	20.8039	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	5.58
	25.9821	Squalene	$C_{30}H_{50}$	410.7	5.53
	25.0025	γ-Tocopherol	$C_{28}H_{48}O_2$	416.7	4.41
100 Gy	18.8120	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	3.99
	15.3272	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	151.12	19.37
	26.1497	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354.5	11.99
	24.4819	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5	8.53
	25.4250	Stigmasterol	$C_{29}H_{48}O$	412.7	8.29
	20.8258	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	6.67
	26.7979	γ-Sitosterol	$C_{29}H_{50}O$	414.7	4.55
	25.9895	Squalene	$C_{30}H_{50}$	410.7	4.02
	18.8303	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	4.82
	20.2760	9,11-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.5	4.34
	26.0332	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.6	2.74

Table 2. List of the most abundant phytochemicals with their functions (If any)

Compound	Functions	Reference
1,3-Propanediol,2-ethyl-2-(hydroxymethyl)-	Antibacterial, anti-inflammatory and antifungal	(7)
n-Hexadecanoic acid	Anti-androgenic, antioxidant, pesticide activities and flavouring agent	(8, 9)
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Antioxidant and antimicrobial	(10)
9,12-Octadecadienoic acid (Z,Z)-	Antibacterial	(11, 12)
Hexadecanoic acid, methyl ester	Antioxidant and anti-inflammatory	(13)
Squalene	Chemopreventive substance for cancer and application in cosmetic industries	(14, 15)
Glycerol 1-palmitate	Anti-diabetic	(16)
Octadecanoic acid	Antibacterial and antifungal	(17)
Cis-13-octadecenoic acid	Decrease blood cholesterol levels and insulin	(18)
9-Octadecenoic acid (Z)-, methyl ester	Antioxidant	(10)
1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	Anti-fungal	(19)
Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	Anti-microbial	(20)
Stigmasterol	Anti-cancer, anti-inflammatory, anti-diabetic and antioxidant	(21)
γ-Sitosterol	Anti-inflammatory, anti-asthma activities and anti-cancer	(22, 23)
9,11-Octadecadienoic acid,methyl ester, (E,E)-	Larvicide and ovicide	(24)
Octadecanoic acid, 2,3-dihydroxypropyl ester	Anticancer and antimicrobial	(20)
γ-tocopherol	Antioxidant, anti-inflammatory	(24, 25)

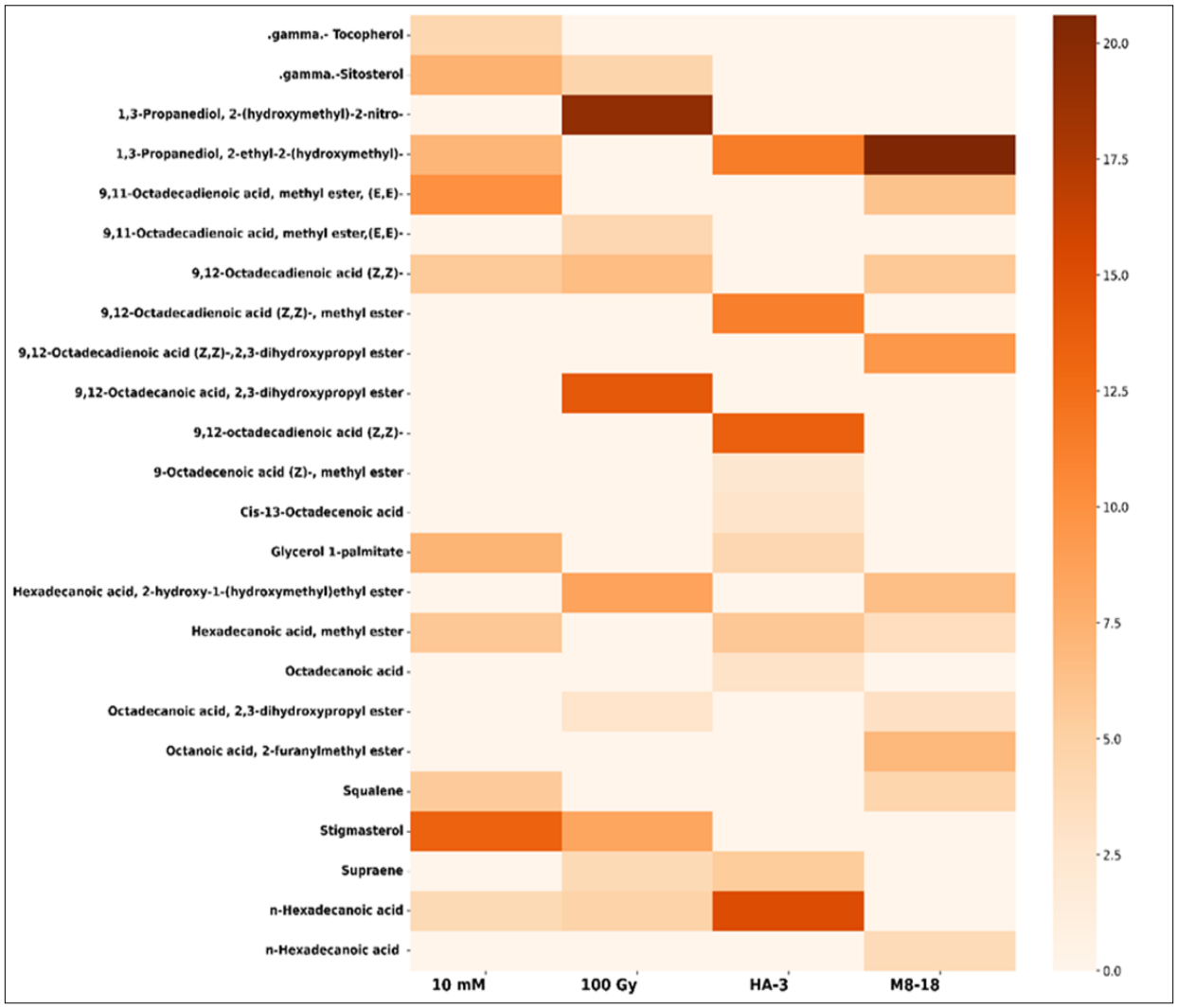

Fig. 1. Heat map showing various compound compositions in HA-3 and its mutants.

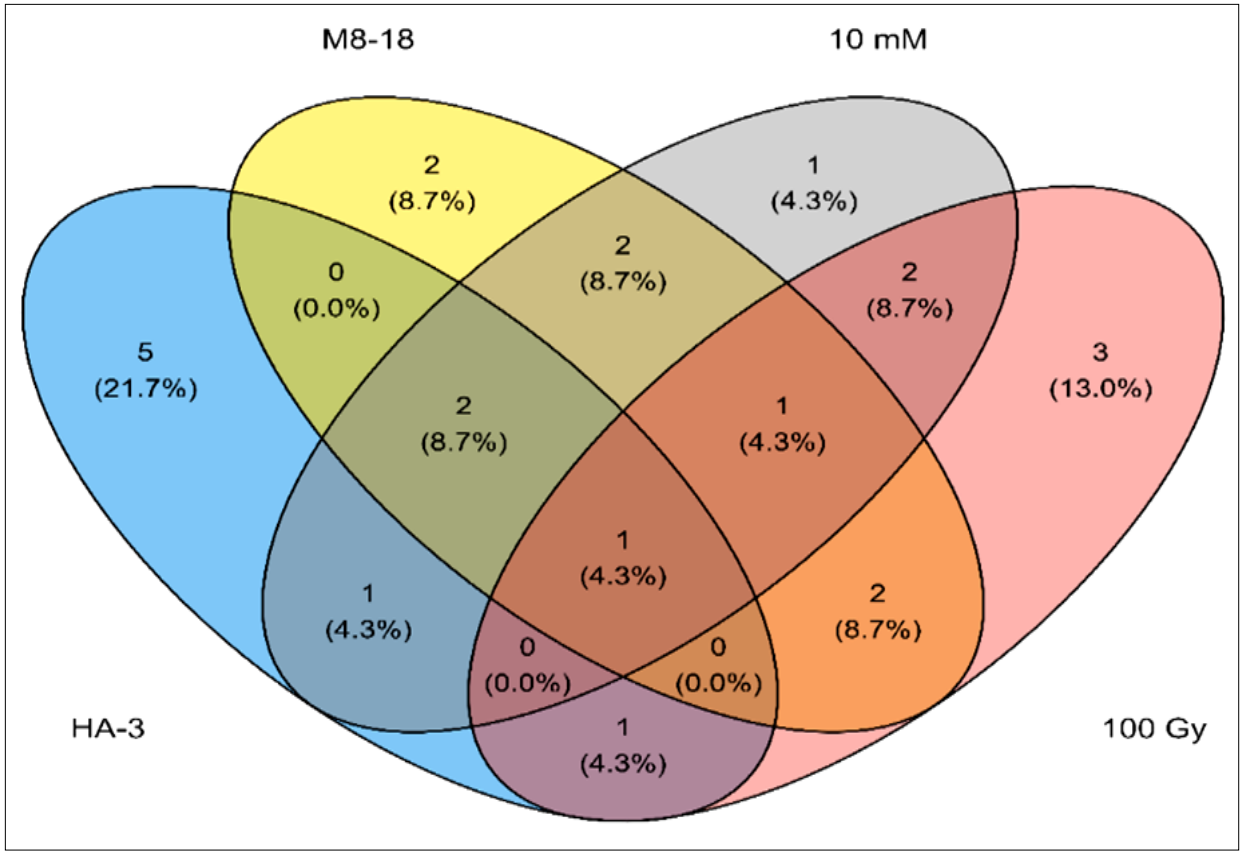

Fig. 2. Venn diagram showing various phytochemicals relations between HA-3 and its mutants.

significant metabolic changes, leading to the production of new phytochemicals.

n-hexadecanoic acid, also known as palmitic acid, has been documented to function as an anti-inflammatory agent (26, 27). Stigmasterol, a steroidal compound used as a precursor for vitamin D3, possesses antimicrobial, anticancer, anti-arthritic, diuretic and anti-inflammatory properties. It is also beneficial in preventing certain cancers, including ovarian, prostate, breast and colon cancers (28, 29). Squalene is a polyunsaturated hydrocarbon naturally found in plants and serves as a precursor for various bioactive compounds such as cholesterol, sterols, hormones, vitamins and triterpenoids across a range of organisms. It has applications in cancer treatment and lowering blood cholesterol and triglycerides. Due to its significance in cosmetics, nutraceuticals, pharmaceuticals and the automotive industry, the demand for squalene is high (30, 31). Gamma-tocopherol (γ-T), the predominant form of vitamin E in seeds, has received increasing attention for its health benefits. γ-T has demonstrated antioxidant properties in both food and *in vitro* studies, showing enhanced efficacy in trapping lipophilic electrophiles and reactive nitrogen and oxygen species (32). *Cis*-13-octadecenoic acid methyl ester is a fatty acid methyl ester with therapeutic applications in medicine and surgery (33). Octadecanoic acid, 2,3-dihydroxypropyl ester, a member of the monoacylglycerol family, functions as a food additive and exhibits antimicrobial and anticancer properties (34). Gamma-sitosterol exhibits anti-inflammatory and anti-asthmatic properties (35). The HA-3 mutants underwent significant metabolic alterations, creating new phytochemicals with diverse and potentially beneficial biological activities. These findings significantly expand our understanding of HA-3 and its mutants' metabolic diversity and highlight its potential as a source of novel bioactive compounds.

Conclusion

In the present GC-MS analysis, abundant information was retrieved regarding the phytochemicals present in HA-3 and its mutants. Significant differences in phytochemicals were observed between HA-3, its mutants and among the mutants themselves. Specifically, HA-3 contained 32 phytochemical compounds, while M8-18 exhibited 44 compounds, 10 mM contained 32 compounds and 100 Gy comprised 36 compounds. Additionally, newly identified phytochemicals in were presents in the mutants but absent in HA-3. These newly identified phytochemicals exhibit various bioactive properties such as antifungal, antioxidant, anticancer and anti-inflammatory activities. Therefore, the HA-3 mutants possess novel phytochemicals with potential applications in pharmaceuticals and nutritional consumption.

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Authors' contributions

SB carried out the experiment, recorded observations and analysed the data. SA guided the research by formulating the research concept and assisted in writing the final manuscript. ATH designed and coordinated the experiment, as well summarized and revised the manuscript. APS contributed to the statistical analysis and interpretation of the findings. SP participated in the sequence alignment and provided guidance for recording observations. All the authors were read and approve the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Dhivyabharathi P, Rajasree V, Devi H, Thiruvengadam V. Genetic diversity studies in lablab (*Lablab purpureus* L.) genotypes. Elect J Plant Breeding. 2019;10(2):717–19. https://doi.org/10.5958/0975-928x.2019.00092.9
2. Bello-Pérez LA, Sáyago-Ayerdi SG, Chávez-Murillo CE, Agama-Acevedo E, Tovar J. Proximal composition and *in vitro* digestibility of starch in lima bean (*Phaseolus lunatus*) varieties. J Sci Food Agric. 2007;87(14):2570–75. https://doi.org/10.1002/jsfa.3005
3. Naeem M, Shabbir A, Ansari AA, Aftab T, Khan MMA, Uddin M. Hyacinth bean (*Lablab purpureus* L.)-An underutilised crop with future potential. Sci Hortic. 2020;272:109551. https://doi.org/10.1016/j.scienta.2020.109551
4. Prada J, Padmaja V. Studies on yield, quality and physiological parameters in induced mutant population of M_2 generation in field bean [*Lablab Purpurea var. lignosus* (L.) Prain]. Pharma Innov J. 2022;11(9):1711-15.
5. Sowmya P, Sankari A, Hemavathy AT, Usha Nandhini Devi H, Iyanar K, Pugalendhi L, et al. Effect of gamma irradiation and EMS on germination, seedling length and seedling vigour index in vegetable mochai (*Lablab purpureus var. lignosus* L.). Pharma Innov J. 2023;12(9):2316-19.
6. Bari T, Saeed S, Tayyab M, Anjum AA, Mehmood T. GC-MS bioactives profiling, antibacterial and cytotoxic potential of jamun (*Syzygium cumini* L.) extracts against food-borne pathogen *Salmonella enteritidis*. Pharmaceut Chem J. 2024;57(10):1586-92.
7. Alagbe JO. Bioactive compounds in ethanolic extract of *Strychnos innocua* root using gas chromatography and mass spectrometry (GC-MS). Drug Discov. 2023;17:e4dd1005. https://doi.org/10.54905/disssi.v17i39.e4dd1005
8. Elaiyaraja A, Chandramohan G. Comparative phytochemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. J Pharmacogn Phytochem. 2016;5(6):158–71.
9. Ponnamma S, Manjunath K. GC-MS analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (Nees) T. Anders. Int J Pharm Bio Sci. 2012;3(3):570–76.
10. Rahman M, Ahmad S, Mohamed M, Ab Rahman M. Antimicrobial compounds from leaf extracts of *Jatropha curcas, Psidium guajava* and *Andrographis paniculata*. Sci World J. 2014;2014 (1):635240. https://doi.org/10.1155/2014/635240
11. Dilika F, Bremner P, Meyer J. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: A plant used during circumcision rites. Fitoterapia. 2000;71(4):450–52. https://doi.org/10.1016/s0367-326x(00)00150-7

12. McGaw L, Jäger A, Van Staden J. Isolation of antibacterial fatty acids from *Schotia brachypetala*. Fitoterapia. 2002;73(5):431–33. https://doi.org/10.1016/s0367-326x(02)00120-x

13. Hema R, Kumaravel S, Alagusundaram K. GC/MS determination of bioactive components of *Murraya koenigii*. J Am Sci. 2011;7 (1):80–83.

14. Huang ZR, Lin YK, Fang JY. Biological and pharmacological activities of squalene and related compounds: Potential uses in cosmetic dermatology. Molecules. 2009;14(1):540–54. https://doi.org/10.3390/molecules14010540

15. Smith TJ. Squalene: Potential chemo preventive agent. Expert Opin Investig Drugs. 2000;9(8):1841–48. https://doi.org/10.1517/13543784.9.8.1841

16. Lawal M, Verma AK, Umar IA, Gadanya AM, Umar B, Yahaya N, et al. Analysis of new potent anti-diabetic molecules from phytochemicals of *Pistia strateotes* with Sglt1 and G6pc proteins of *Homo sapiens* for treatment of diabetes mellitus. An *in silico* approach. IOSR J Pharm Biol Sci. 2020;15:59–73.

17. Gopalakrishnan K, Udayakumar R. GC-MS analysis of phytocompounds of leaf and stem of *Marsilea quadrifolia* (L.). Int J Biochem Res Rev. 2014;4(6):517–26. https://doi.org/10.9734/ijbcrr/2014/11350

18. Ojewumi M, Obanla O, Taiwo S, John A. Phytochemical screening and microbial assessment of *Moringa oleifera* seed crude oil extract. Rasayan J Chem. 2021;14:1835–44. https://doi.org/10.31788/rjc.2022.1516543

19. Popendorf W, Selim M, Lewis MQ. Exposure while applying industrial antimicrobial pesticides. Am Ind Hyg Assoc J. 1995;56 (10):993–1001. https://doi.org/10.1080/15428119591016421

20. Arora S, Kumar G. Phytochemical screening of root, stem and leaves of *Cenchrus biflorus* Roxb. J Pharmacogn Phytochem. 2018;7(1):1445–50.

21. Bakrim S, Benkhaira N, Bourais I, Benali T, Lee LH, El Omari N, et al. Health benefits and pharmacological properties of stigmasterol. Antioxidants. 2022;11(10):1912. https://doi.org/10.3390/antiox11101912

22. Naikwadi PH, Phatangare ND, Mane DV. Active anti-inflammatory potency of γ-sitosterol from *Woodfordia floribunda Salisb*. J Plant Sci Res. 2022;38(2):1-9. https://doi.org/10.32381/JPSR.2022.38.02.4

23. Sianipar NF, Purnamaningsih R. Enhancement of the contents of anticancer bioactive compounds in mutant clones of rodent tuber (*Typhonium flagelliforme Lodd*.) based on GC-MS analysis. Pertanika J Trop Agric Sci. 2018;41(1):305-20.

24. Jiang Q, Ames BN. γ-Tocopherol, but not α-tocopherol, decreases proinflammatory eicosanoids and inflammation damage in rats. FASEB J. 2003;17(8):816–22. https://doi.org/10.1096/fj.02-0877com

25. Wagner KH, Kamal-Eldin A, Elmadfa I. Gamma-tocopherol–an underestimated vitamin? Ann Nutr Metab. 2004;48(3):169–88. https://doi.org/10.1159/000079555

26. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of *n*-hexadecanoic acid: Structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80 (3):434–39. https://doi.org/10.1111/j.1747-0285.2012.01418.x

27. Afolayan FI, Odeyemi RA, Salaam RA. *In silico* and *in vivo* evaluations of multistage anti plasmodial potency and toxicity profiling of n-hexadecanoic acid derived from *Vernonia amygdalina*. Front Pharmacol. 2024;15:1445905. https://doi.org/10.3389/fphar.2024.1445905

28. Kametani T, Furuyama H. Synthesis of vitamin D3 and related compounds. Med Res Rev. 1987;7(2):147–71. https://doi.org/10.1002/med.2610070202

29. Kumari K, Kumar A, Oraon V. Phytochemical and GC-MS analysis of *Cuscuta reflexa* Roxb: A parasitic plant collected from host *Vitex negundu.* Ann Plant Soil Res. 2024;26(2):449-55. https://doi.org/10.47815/apsr.2024.10383

30. Senbagalakshmi P, Muthukrishnan S, Jebasingh T, Kumar TS, Rao MV, Senbagalakshmi P. Squalene, biosynthesis and its role in production of bioactive compounds, a proper scientific challenge - A Review. J Emerg Technol Innov Res. 2019;6(2):505-26.

31. Song Y, Yang X, Li S, Luo Y, Chang JS, Hu Z. Thraustochytrids as a promising source of fatty acids, carotenoids and sterols: Bioactive compound biosynthesis and modern biotechnology. Crit Rev Biotech. 2024;44(4):618-40. https://doi.org/10.1080/07388551.2023.2196373

32. Suganandam K, Jeevalatha A, Kandeepan C, Kavitha N, Senthilkumar N, Sutha S, et al. Profile of phytochemicals and GCMS analysis of bioactive compounds in natural dried seed removed ripened pods methanolic extracts of *Moringa oleifera*. J Drug Deliv Ther. 2022;12(5-S):133–41. https://doi.org/10.22270/jddt.v12i5-s.5657

33. Awonyemi O, Abegunde M, Olabiran T. Analysis of bioactive compounds from *Raphia taedigera* using gas chromatography-mass spectrometry. Eur Chem Commun. 2020;2(8):933–44.

34. Janani SR, Singaravadivel K. Screening of phytochemical and GC-MS analysis of some bioactive constituents of *Asparagus racemosus*. Int J Pharm Tech Res. 2014;6(2):428–32.

35. Sharma A, Kumar V, Kohli SK, Thukral AK, Bhardwaj R. Phytochemicals in *Brassica juncea* L. seedlings under imidacloprid-epibrassinolide treatment using GC-MS. J Chem Pharm Res. 2015;7(10):708–11.