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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(11): 1537-1544

www.thepharmajournal.com
Received: 14-09-2021
Accepted: 23-10-2021

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Comparative analysis of metabolomics associated with intra-varietal graft of dioecious papaya var. TNAU Papaya CO 8

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Abstract
Carica papaya Linn. Belongs to the family caricaceae and TNAU Papaya CO.8 is a dioecious, red fleshed variety with high yield and well suitable for tropical conditions and propagated through seeds. The main constraint in production is, it is dioecious in nature i.e. produces male (staminate) and female (pistillate) plants separately. Hence, grafting technology has been standardized to produce separate male and female plants for easy propagation. Though the success rate of grafts was reported to be more than 80 per cent, at times during unfavorable climatic conditions the failure in graft union occurs. Hence, in the present study GC-MS analysis was done to find out the secondary metabolites associated with the successful grafts compared to the unsuccessful grafts. The GC-MS analysis revealed that trifluoroacetoxy pentadecanal, benzoyl-2-hydroxyadamantane (Vildagliptin), styrene, Nonanal, dodecane, cyclopentylpropionic acid, tetradecane, hexdecane, hexdecanoic acid, octadecanedioic acid, pentatriacontene, tetradecynoic acid, vaccenic acid, squalene and isopropyl linoleate were the compounds present in successful grafts. Among the 15 compounds, six compounds *viz.,* tetradecane, dodecane, styrene, hexadecane, hexadecanoic acid and nonanal were reported to possess plant growth promoting activity, which might have contributed for the successful graft union. Also organic compounds like lipid and lipid like compounds were higher in the successful grafts. Benzenoids indicated a negative impact on the graft union. This compound was observed to be present in higher amount in the unsuccessful grafts as compared to successful grafts that might have hindered graft union. A detailed study of the mode and mechanism of action of these compounds will give a clear understanding of their involvement in graft union.

Keywords: Papaya, GC-MS, grafted plants, metabolite profiling

Introduction
Carica papaya L. (Caricaceae) is a herbaceous perennial tree mainly cultivated for its delicious fruits, across the tropical countries. Sexual propagation is the common method to perpetuate the papaya. TNAU Papaya CO 8 is a red fleshed variety with high yield and well suited for cultivation under tropical conditions which is released from TNAU, Coimbatore. Since it is a dioecious variety, equal population of male and female plants will be produced, which is the only constraint regarding the cultivation of this variety. Rouging have to be done in order to maintain the optimum male and female population for economic yield which consumes excess time and labour. Asexual propagation methods can be substituted over sexual propagation to surmount these problems. Grafting is one of the promising technology in dioecious papaya to produce male and female plants separately, overcoming the difficulty through seed propagation. Grafts have several advantages over seedlings like precocity in flowering, besides dwarf bearing and long life span. Among asexual propagation methods, grafting technique is found to be more effective and successful for papaya. Hence, in dioecious varieties through grafting technique, desirable sex form *i.e* pistillate or staminate plants can be produced by collecting scions from the respective plants immediately after flowering. Allan *et al*., (2010) [2] reported that in papaya, higher percentage of success was obtained by side grafting, whereas Senthil kumar *et al.* (2011) [25] reported that cleft grafting was superior over side grafting. In the present study, the highest survival percentage was recorded (93.33%) in the month of September, 2010 with cleft grafting on 3 months old rootstock. which implies the influence of seasonal variation on graft success percentage. Hence, the present study based on the comparison of success and failure grafts in terms of metabolomics was proposed.

Materials and Methods

Cleft grafting in TNAU Papaya CO 8 variety

The papaya variety, TNAU Papaya CO 8 plants were raised in an insect proof net house in order to get the scions free of PRSV inoculum and the rootstocks were raised in nursery under shade net house condition. Cleft method of grafting was adopted where (Senthil kumar *et al.*, 2011; Akino *et al*., 2016) [25, 1] the scion was taken from 25-30 days old lateral shoot with 8-10 cm length, and the leaves of the scion were removed with a sterile and sharp knife and given a wedge-shaped cut of three to four cm length. Three to four-month old seedling was chosen as rootstock, and a straight cut was made at a height of 10 centimeters and then the rootstock was cut vertically giving a V-shape cut. The scion was carefully inserted into the V-shaped cut of the rootstock and firmly tied with a polythene strip and covered with polyethylene sleeves. The grafts were then shifted to a mist chamber, where the relative humidity was maintained at 80-90 percent, which was a standardized conductive atmosphere for graft success.

Characterization of volatile and non-volatile compounds in the scion, rootstock, successful grafts and unsuccessful grafts

The grafts union of size 5-6 cm were taken from the nursery then air dried at room temperature and pulverized for characterization. The extraction of different metabolites in the scion, rootstock, successful graft and unsuccessful grafts (Fig.1) were determined adopting the procedure of Selvamangai *et al.* (2012) [23]. Fresh plant samples weighing around 10 g from the graft union of about 5 cm length were taken and dried under shade and pulverized. The powdered plant samples were dissolved in 100 ml HPLC grade methanol and shaken for 42 hours at 170 rpm in an orbital shaker. The extracts were filtered using Whatman No.1 filter paper (Selvamangai *et al.,* 2012) [23] and they were characterized through GC- MS Perkin Elmer clarus SQ8C DB-5 MS capillary standard non polar column.

Results and Discussion

Volatile compounds of rootstocks, scion, successful and unsuccessful grafts

The present study was carried out for the identification of associated biomolecules or metabolites from rootstocks, scion, successful graft and unsuccessful graft through GC-MS analysis. The study revealed the presence of diversity of biomolecules in different samples. There were 15 active biomolecules identified in the rootstock (Fig.3; Table 1) *viz.*, (2-mercaptoethyl) guanidine, tridcanal, methyl 4,6 decadienyl ether, hexadecanoic acid, Furaldehyde phenylhydrazone, 1h-indene, pentadecanal, hexadecene, butyl-4-hydroxybenzaldehyde, dodecane, hexadecenal, cyclopropanebutanoic acid, oleic acid, myristynoyl pantetheine and nizatidine. In scion, 15 biomolecules compounds were *viz.*, identified tetradecane, hexadecane, hexadecanoic acid, octadecenoic acid, piperidinone, neronine, dasycarpidan-1-methanol, neophytadiene, oleic acid, dodecane, nonanal, decadienal, dimethoxy benzoquinone, benzenedicarboxylic acid (Fig.4; Table 2). In the unsuccessful grafts, 14 biomolecules were identified and they were dodecane, dihydroxycoumarin-4-acetic acid, phthalic acid, hexadecanoic acid, diisooctyl phthalate, bis (2-ethylhexyl) phthalate, tetradeacne, octen, nonanal, oxirane, decadienal, myristynoyl pantetheine, oleic acid, neophytadiene (Fig.5; Table 3). On the other hand, 17 biomolecules were identified in successful grafts *viz.*, trifluoroacetoxy pentadecane, benzoyl-2-hydroxyadamantane (vildagliptin), styrene, cyclopentylpropionic acid, octadecanedioic acid, dodeacne, pentatriacontene, tetradecynoic acid, vaccenic acid, squalene, isopropyl linoleate, hexadecanoic acid, benzenedicarboxylic acid, nizatidine, tetradecane, nonanal and hexadecane (Fig.6; Table 4)

Oleic acid was found to be common in rootstock, scion and unsuccessful graft, whereas myristynoyl pantetheine was common in root stock and unsuccessful grafts. Nizatidine was found to be common in rootstock and unsuccessful graft whereas neophytadiene compound was observed in both scion and unsuccessful graft. The compounds like nonanal, decadienal were found in all the samples except rootstock. Dimethoxy benzoquinone and benzenedicarboxylic acid was found in both scion and successful graft. This difference in volatiles between the scion and rootstock may be due to the different growing conditions and age, where the mother plant from which the scion is taken is older than the rootstock. As the mother plant from where the scion is taken is older exposing it to different environmental conditions compared to the rootstock.

Diiscootyl phthalate which is a autotoxic compound was present at a concentration of 22.7% in the unsuccessful graft that might be one of the reasons for graft incompatibility and it is in accordance with the findings of Jia-Jun *et al*. (2017). It was reported that diiscootyl phthalate is a major autotoxic agent in tobacco root exudates that affect the seed germination and seedling growth and cause tobacco autotoxicity. Many autotoxins like diiscootyl phthalate has been found to inhibit the seed germination, seedling growth, nutrient uptake, cell division and cytoskeleton formation, photosynthesis, reactive oxygen species generation and gene expression in many plants (Jia-Jun *et al*., 2017).

The metabolites like tetradecane, hexdecane, dodecane, hexadeconoic acid, styrene, and nonanal have been discovered as growth promoting volatiles in plants by Jishma *et al*. (2017) [12] and in the present study, all these compounds were found in the successful grafts that would have been responsible for the graft union. The concentration of these compounds in the successful grafts, showed elevated peak areas compared to unsuccessful grafts. In the successful grafts, the concentration of tetradecane, dodecane, hexadecanoic acid and nonanal were 1.693%, 0.859%, 6.825%, and 0.492% respectively and on the otherhand, it was 0.342%, 0.247%, 2.872%, and 0.256% respectively in unsuccessful grafts. The hexadecane and styrene compounds were absent in the unsuccessful graft. Hence the study reveals that the growth promotion metabolites like tetradecane, dodecane, hexadecanoic acid, nonanal, hexadcane and styrene played a significant role in the graft union which should be characterized based on its mode and mechanism of action.

Organic compound composition in rootstocks, scion, unsuccessful graft and successful graft

The organic compound composition varied in different samples of rootstock, scion, unsuccessful and successful grafts. TNAU papaya CO.8 rootstock (Fig.2. a) contains 46 per cent of lipids and lipid-like chemicals, 13 per cent of organoheterocyclic compounds and hydrocarbons, 7 per cent of organic oxygen compounds, organic nitrogen compounds, benzenoids, organic acids and derivatives. Scion (Fig.2. b) comprised of 31 per cent lipids and lipid-like substances, 23 per cent organic oxygen compounds, 23 per cent hydrocarbons, 15 per cent of benzenoids and 8 per cent organ heterocyclic compounds. The unsuccessful grafts recorded 14 per cent of organic oxygen compounds, 22 per cent of lipids and lipid-like molecules, 22 per cent of benzenoids, 7 per cent of organ heterocyclic compounds, phenylpropanoids, polyketides, organic acids and derivatives were found in the (Fig.2. c), whereas the successful grafts had 41 per cent lipids and lipid-like compounds, 12 per cent of benzenoids, organic acids and derivatives, 6 per cent of organic oxygen compounds and 6 per cent of organ heterocyclic compounds and 23 per cent of hydrocarbons (Fig.2. d). Hence, the present study gives a deep insight on the role of lipid and lipid like chemicals in the graft union and the benzenoid compounds involvement in the incompatibility of scion and stock.

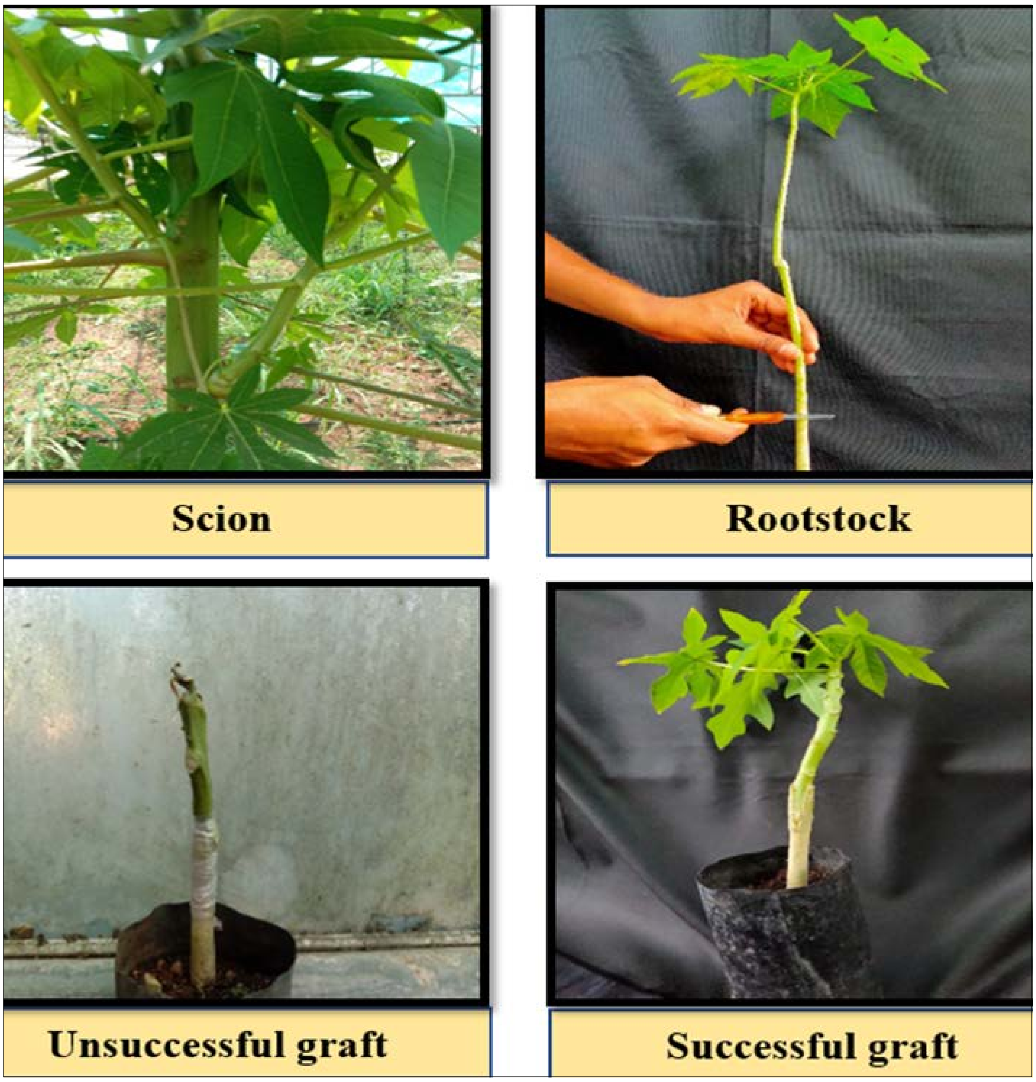


Fig 1: Cleft grafting and graft union in TNAU papaya CO 8 variety

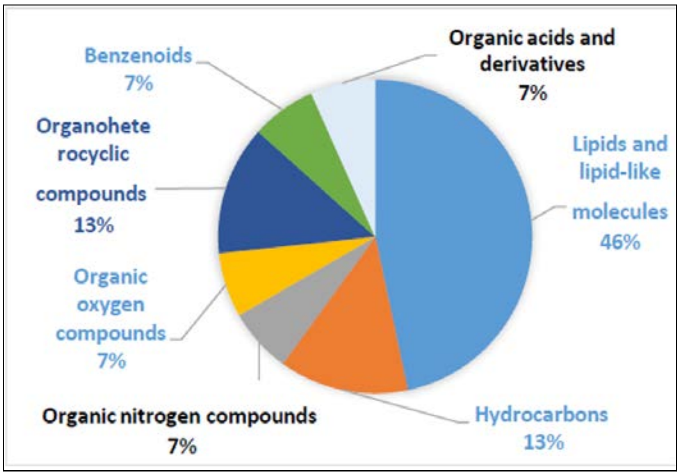


Fig 2a: Rootstock

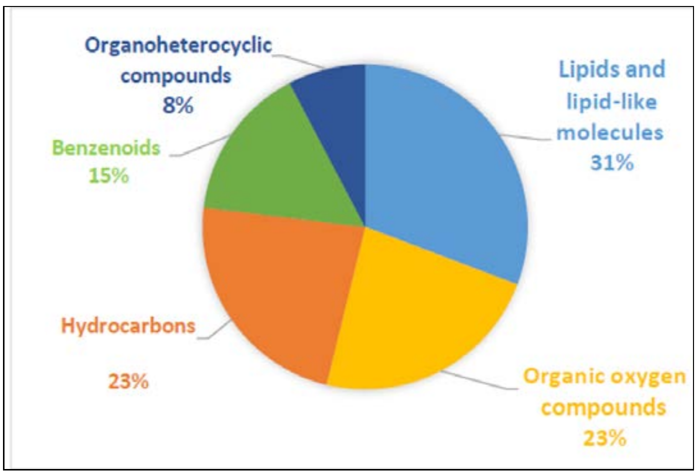
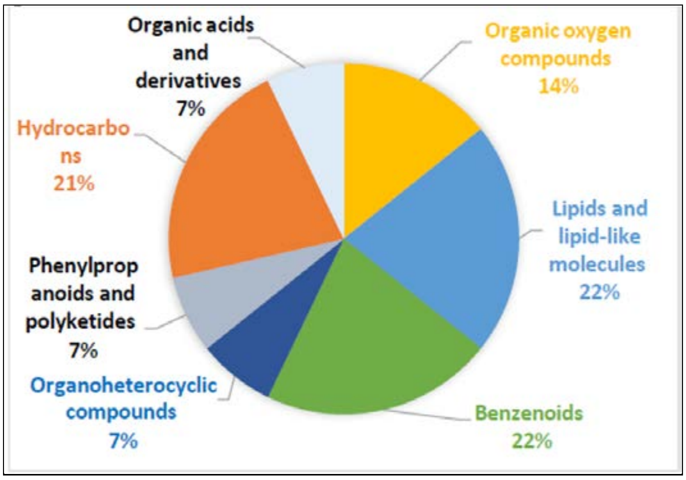


Fig 2b: Scion



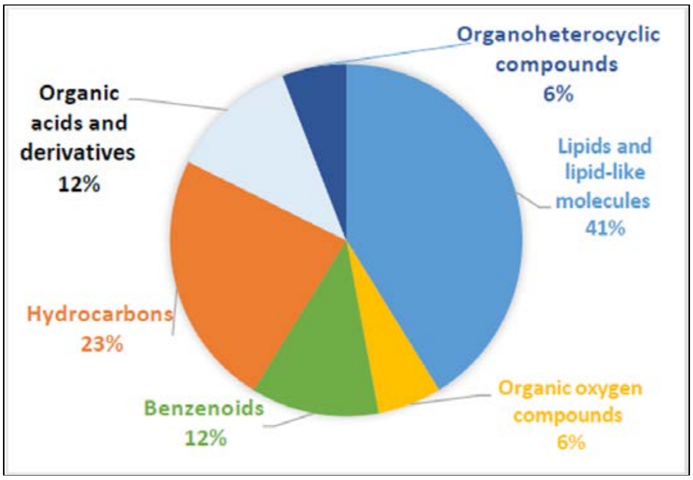


Fig 2c: Unsuccessful graft **Fig 2d:** Successful graft

Fig 2: Comparative profiling of metabolites in the scion, rootstock, successful and unsuccessful grafts

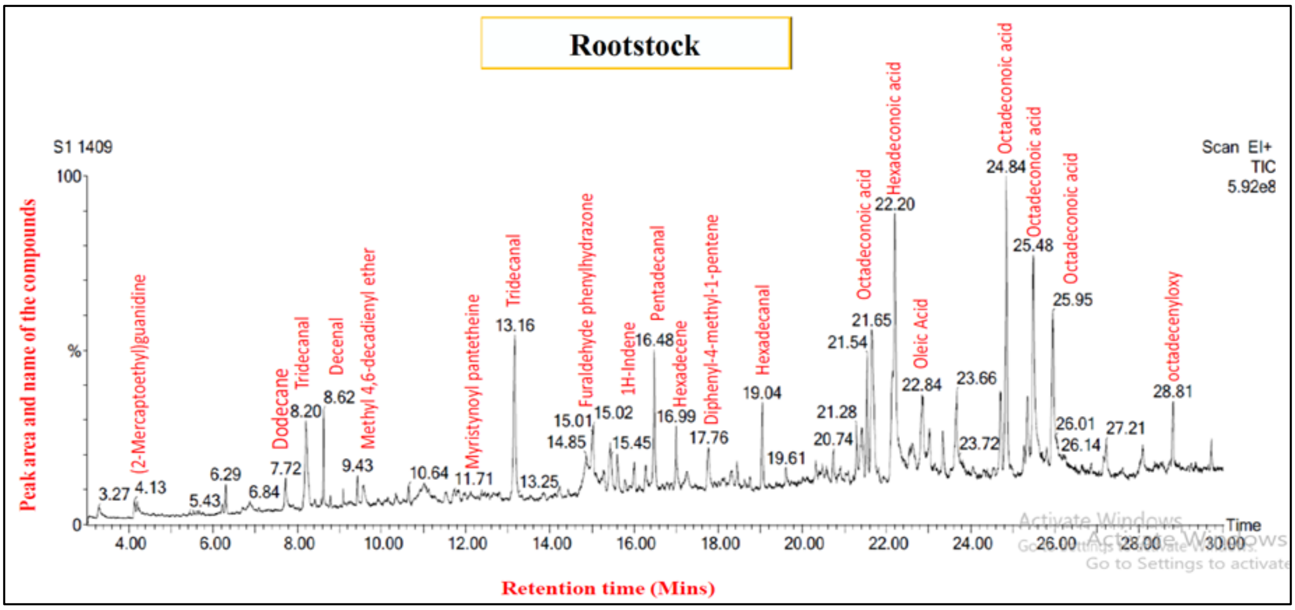


Fig 3: Gas Chromatography Mass Spectrometry (GC-MS) profiling in the rootstocks

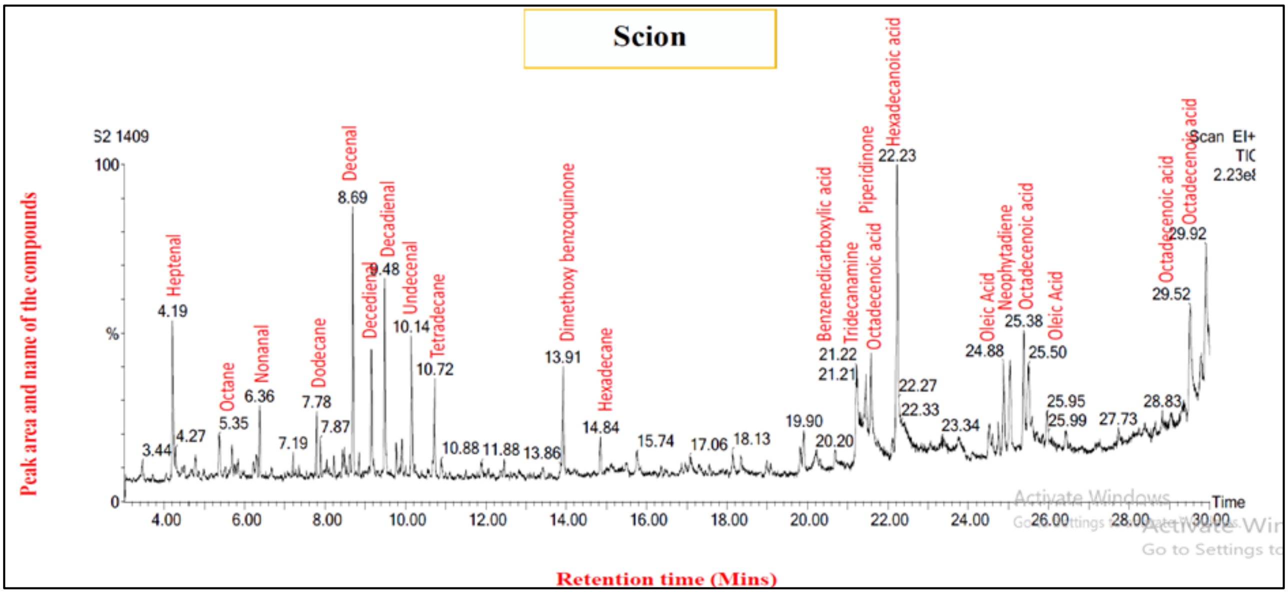


Fig 4: Gas Chromatography Mass Spectrometry (GC-MS) profiling in the scion

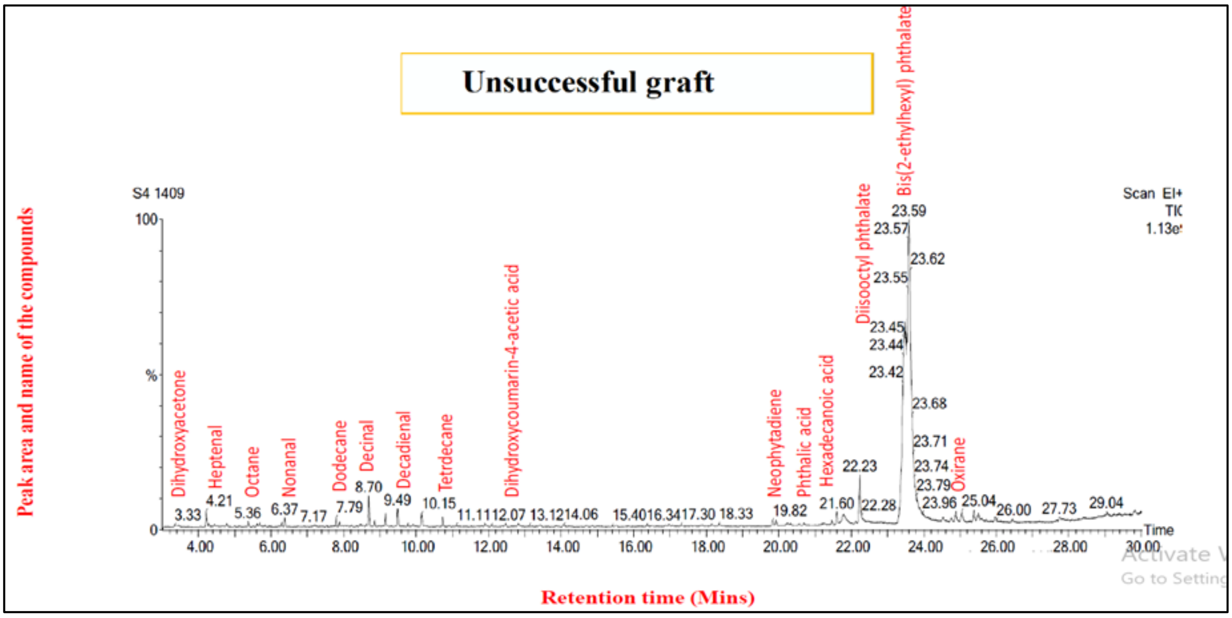


Fig 5: Gas Chromatography Mass Spectrometry (GC-MS) profiling in the unsuccessful graft

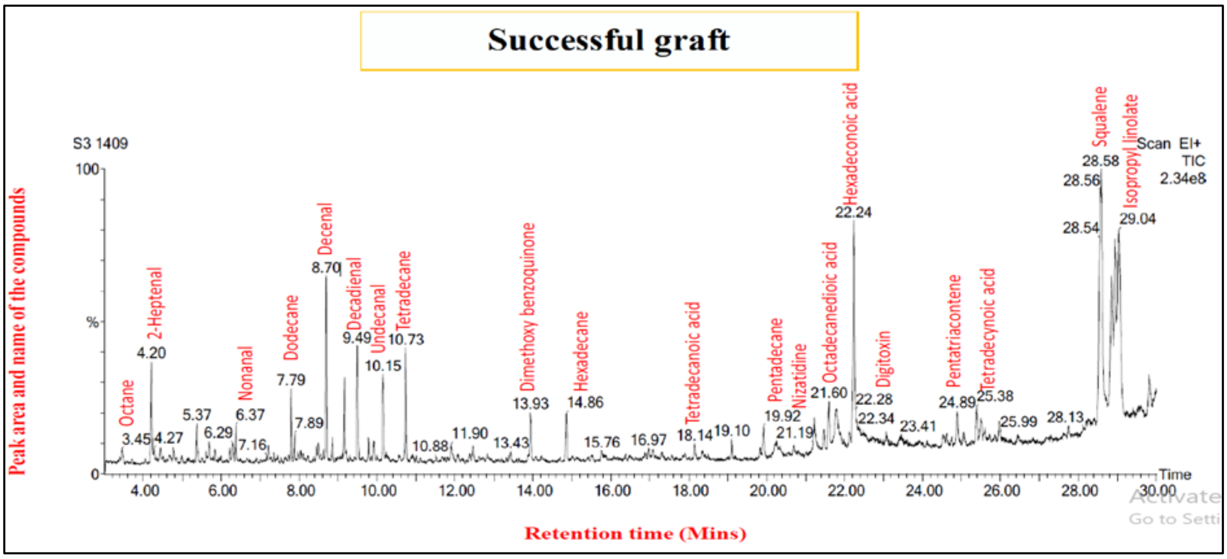


Fig 6: Gas Chromatography Mass Spectrometry (GC-MS) profiling in the successful graft

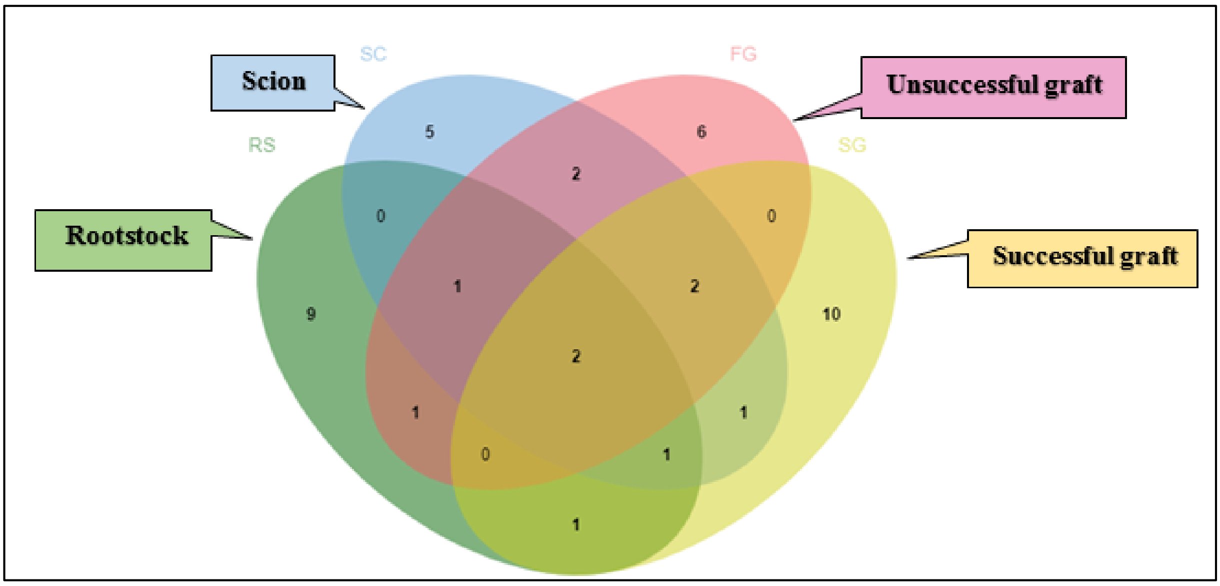


Fig 7: Venn Diagram of GC-MS analysis

Table 1: Metabolites identified from the active fraction of methanol extract of rootstocks of TNAU papaya CO 8 by GC-MS analysis

Peak	Retention time	Area %	Compound	Activity	Reference
1.	4.189	0.487	(2- Mercaptoethyl) guanidine	No activity reported	-
2.	7.715	0.712	Dodeacne	Plant growth promotion	Jishma *et al.* 2017 [12]
3.	8.210	2.414	Tridecanal	Anti-microbial	Yucel *et al.* 2017
4.	9.576	0.521	Methyl 4,6-decadienyl ether	No activity reported	-
5.	11.117	0.483	Myristynoyl pantetheine	Growth stimulant	Nisha *et al.* 2020
6.	15.023	2.627	Furaldehyde phenylhydrazone	No activity reported	-
7.	15.433	1.152	1H-Indene	Anti-fungal	Akhter *et al.* 2019
8.	16.269	0.485	Nizatidine	No activity reported	-
9.	16.004	0.534	Pentadecanal	Anti-microbial	CHEBI (17302)
10.	17.269	0.676	Butyl-4-hydroxybenzaldehyde	Antioxidant	Zhao *et al.* 2020
11.	20.735	0.635	Hexadecenal	No activity reported	-
12.	21.406	1.058	Cyclopropane butanoic acid	No activity reported	-
13.	22.196	8.660	Hexadecanoic acid	Plant growth promotion	Jishma *et al.*,2017 [12]
14.	22.841	2.129	Oleic Acid	Anti-bacterial	Dilika *et al.* 2000
15.	23.011	1.11	Hexadecane	Plant growth promotion	Jishma *et al.* 2017 [12]

Table 2: Metabolites identified from the active fraction of methanol extract of scion of TNAU papaya CO 8 by GC-MS analysis

Peak	Retention time	Area %	Compound	Activity	Reference
1.	6.365	0.673	Nonanal	Plant growth promotion	Jishma *et al.* 2017 [12]
2.	7.780	0.596	Dodecane	Plant growth promotion	Jishma *et al.* 2017 [12]
3.	9.476	2.031	Decadienal	Potent botanical nematicidal agents	Caboni *et al.* 2012
4.	10.717	1.365	Tetradecane	Plant growth promotion	Jishma *et al.* 2017 [12]
5.	13.913	1.985	Dimethoxy benzoquinone	Antibacterial	Nishina *et al.*1993
6.	14.843	0.517	Hexadecane	Plant growth promotion	Jishma *et al.*2017 [12]
7.	20.220	0.716	Benzenedicarboxylic acid	Antimicrobial	Zayed *et al.* 2019.
8.	22.231	8.557	Hexadecanoic acid	Plant growth promotion	Jishma *et al.* 2017 [12]
9.	21.301	0.824	Octadecenoic acid	Anti-microbial activity	Zhong-hui *et al.* 2010
10.	21.451	2.901	Piperidinone	-	-
11.	24.877	1.597	Oleic Acid	Anti-bacterial	Dilika *et al.* 2000
12.	25.037	1.871	Neophytadiene	Antimicrobial,	Mahalakashmi and Thangapandian (2018)
13.	25.593	0.705	Neronine	-	-
14.	22.551	0.470	Dasycarpidan-1-methanol	Anti-microbial	Rubaye, *et al.* 2017

Table 3: Metabolites identified from the active fraction of methanol extract of unsuccessful graft of TNAU papaya CO 8 by GC-MS analysis

Peak	Retention time	Area %	Compound	Activity	Reference
1.	3.048	0.211	Myristynoyl pantetheine	-	-
2.	5.369	0.220	Octen	Antimicrobial	Vankoten *et al.* 2016
3.	6.375	0.256	Nonanal	Antifungal	Miguel *et al.* 2002
4.	7.790	0.274	Dodeacane	Plant growth promotion	Jishma *et al.* 2017 [12]
5.	9.156	0.400	Decadienal	Potent botanical nematicidal agents	Caboni *et al.* 2012
6.	10.726	0.342	Tetradecane	Plant growth promotion	Jishma *et al.* 2017 [12]
7.	12.812	0.295	Dihydroxycoumarin-4-acetic acid	Antioxidant	Sarsen *et al.* 2015
8.	19.820	0.296	Neophytadiene	Antimicrobial	Mahalakashmi and Thangapandian (2018)
9.	20.250	0.241	Phthalic acid	Antiviral	Uddin *et al.* 2013
10.	22.231	2.872	Hexadecanoic acid	Plant growth promotion	Jishma *et al.* 2017 [12]
11.	23.476	22.713	Diisooctyl phthalate	Autotoxic to plants	Jia-Jun *et al.* 2017
12.	23.592	45.203	Bis(2-ethylhexyl) phthalate	Anti-microbial and anti-bacterial	Habib and Karim (2009)
13.	24.537	0.270	Oxirane	Anti-microbial	Musa *et al.* 2014
14.	25.497	0.503	Oleic Acid	Anti-bacterial	Dilika *et al.* 2000

Table 4: Metabolites identified from the active fraction of methanol extract of successful grafts of TNAU papaya CO 8 by GCMS analysis

Peak	Retention time	Area %	Compound	Activity	Reference
1.	3.078	0.798	Trifluoroacetoxy pentadecane	Antimicrobial	Hussein *et al.* 2015
2.	3.238	0.439	Benzoyl-2-hydroxyadamantane	-	-
3.	3.389	0.431	Styrene	Plant growth promotion	Jishma *et al.* 2017 [12]
4.	6.375	0.492	Nonanal	Plant growth promotion	Jishma *et al.* 2017 [12]
5.	7.790	0.859	Dodecane	Plant growth promotion	Jishma *et al.* 2017 [12]
6.	9.771	0.420	Cyclopentylpropionic acid	Antioxidant	Sebastian *et al.* 2020
7.	10.731	1.693	Tetradecane	Plant growth promotion	Jishma *et al.* 2017 [12]
8.	14.858	0.921	Hexadecane	Plant growth promotion	Jishma *et al.* 2017 [12]
9.	21.226	1.024	Nizatidine	-	-
10.	21.781	2.058	Octadecanedioic acid	Antimicrobial and Insectifuge	Pradeepa *et al.* 2019
11.	22.121	0.401	Benzenedicarboxylic acid	Antimicrobial	Zayed *et al.* 2019.
12.	22.236	6.825	Hexadecanoic acid	Plant growth promotion	Jishma *et al.* 2017 [12]

13.	24.887	0.859	Pentatriacontene	Antibacterial	Kumar *et al.* 2018
14.	25.382	0.705	Tetradecynoic acid	-	-
15.	25.497	0.644	Vaccenic acid	Anti-bacterial	Semwal *et al.*2018
16.	28.578	11.021	Squalene	Antiviral	Sangeetha *et al.* (2020)
17.	29.814	0.959	Isopropyl linoleate	Antimicrobial	Nand *et al.* 2012

Conclusion

The present study was proposed to understand and characterize the different metabolites involved in the successful graft union in the inter-varietal graft of TNAU papaya CO.8. The study revealed that the composition of volatile compounds and organic compounds like lipid and lipid like substances enhanced or played a significant role in the graft union of rootstock and scion or they promoted the compatibility of the rootstock and scion. Hence, further detailed study on the mode and mechanism of action of volatile and organic compounds will give a clear understanding about its involvement of the compounds in the graft union.

References

1. Akino A. “studies on production and evaluation of intergeneric and intervarietal papaya grafts for growth and PRSV tolerance attributes”. M.sc. Thesis submitted to, Tamil Nadu Agricultural Univ., Coimbatore, 2016.
2. Allan P, Clark C, Laing M. Grafting papayas (*Carica papaya* L.). Acta Hort 2010;851:253-258.
3. Al-Rubaye AF, Kaizal AF, Hameed IH. Phytochemical screening of methanolic leaves extract of *Malva sylvestris*. Int. J Pharmacogn. Phytochem. Res 2017;9(4):537-552.
4. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti‐inflammatory property of n‐hexadecanoic acid: structural evidence and kinetic assessment. Chemical biology & drug design 2012;80(3):434-439.
5. Belakhdar G, Benjouad A, Abdennebi EH. Determination of some bioactive chemical constituents from *Thesium humile* Vahl. J Mater Environ Sci 2015;6(10):2778-2783.
6. Blom D, Fabbri C, Connor EC, Schiestl FP, Klauser DR, Boller T. Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. Environmental microbiology 2011;13(11):3047-3058.
7. Brintha S, Rajesh S, Renuka R, Santhanakrishnan VP, Gnanam R. Phytochemical analysis and bioactivity prediction of compounds in methanolic extracts of *Curculigo orchioides* Gaertn. Journal of Pharmacognosy and Phytochemistry 2017;6(4):192-197.
8. Caboni P, Ntalli NG, Aissani N, Cavoski I, Angioni A. Nematicidal activity of (E, E)-2, 4-decadienal and (E)-2-decenal from *Ailanthus altissima* against Meloidogyne javanica. Journal of Agricultural and food Chemistry 2012;60(4):1146-1151.
9. Dilika F, Bremner PD, Meyer JJM. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: A plant used during circumcision rites. Fitoterapia 2000;71(4):450-452.
10. Habib MR, Karim MR. Antimicrobial and cytotoxic activity of di-(2-ethylhexyl) phthalate and anhydrosophoradiol-3-acetate isolated from *Calotropis gigantea* (Linn.) flower. Mycobiology 2009;37(1):31-36.
11. Ingole SN. Phytochemical analysis of leaf extract of *Ocimum americanum L.* (Lamiaceae) by GCMS method. World Scientific News 2016;37:76-87.
12. Jishma P, Hussain N, Chellappan R, Rajendran R, Mathew J, Radhakrishnan EK. Strain‐specific variation in plant growth promoting volatile organic compounds production by five different Pseudomonas spp. as confirmed by response of *Vigna radiata* seedlings. Journal of applied microbiology 2017;123(1):204-216.
13. Jiajun DENG, Zhang Y, Jiwei HU, Jiaguo JIAO, Feng HU, Huixin LI *et al.* Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination and seedling growth. Pedosphere 2017;27(6):1073-1082.
14. Kumar D, Karthik M, Rajakumar R. GC-MS analysis of bioactive compounds from ethanolic leaves extract of *Eichhornia crassipes* (Mart) Solms. and their pharmacological activities. The Pharma Innovation Journal 2018;7(8):459-462.
15. Mahalakashmi R, Thangapandian V. Gas chromatography and mass spectrometry analysis of bioactive constituents of *Maytenus heyneana* (Roth) Roju & Babu (Celestraceae). Journal of Pharmacognosy and Phytochemistry 2019;8(1):2748-2752.
16. Miguel G, Simoes M, Figueiredo AC, Barroso JG, Pedro, LG, Carvalho L. Composition and antioxidant activities of the essential oils of *Thymus caespititius*, *Thymus camphoratus* and *Thymus mastichina*. Food chemistry 2004;86(2):183-188.
17. Musa AM, Ibrahim MA, Aliyu AB, Abdullahi MS, Tajuddeen N, Ibrahim H. Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). Journal of intercultural ethno pharmacology 2015;4(2):129.
18. Nand PR, Drabu SU, Gupta RK. *In vitro* antibacterial and antioxidant potential of medicinal plants used in the treatment of acne. Int J Pharm Pharm Sci 2012;4(1):185-90.
19. Nishina A, Uchibori T. Antimicrobial activity of 2, 6-dimethoxy-p-benzoquinone, isolated from thick-stemmed bamboo, its analogs. Agricultural and biological chemistry 1991;55(9):2395-2398.
20. Pratheeba T, Taranath V, Gopal DS, Natarajan D. Antidengue potential of leaf extracts of *Pavetta tomentosa* and *Tarenna asiatica* (Rubiaceae) against dengue virus and its vector Aedes aegypti (Diptera: Culicidae). Heliyon 2019;5(11):e02732.
21. Pu ZH, Zhang YQ, Yin ZQ, Jiao XU, Jia RY, Yang L. Antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3, 4-diyl ester from neem oil. Agricultural Sciences in China 2010;9(8):1236-1240.
22. Sangeetha B, Krishnamoorthy AS, Sharmila DJS, Renukadevi P, Malathi VG, Amirtham D. Molecular modelling of coat protein of the Groundnut bud necrosis tospovirus and its binding with Squalene as an antiviral agent: *In vitro* and in silico docking investigations. International Journal of Biological Macromolecules 2021;189:618-634.
23. Selvamangai G, Anusha Bhaskar. GC-MS analysis of

phytocomponents in the methanolic extract of *Eupatorium triplinerve.* Asian Pacific Journal of Tropical Biomedicine 2012, S1329-S1332.
24. Semwal P, Painuli S, Badoni H, Bacheti RK. Screening of phytoconstituents and antibacterial activity of leaves and bark of *Quercus leucotrichophora* A. Camus from Uttarakhand Himalaya. Clinical Phytoscience 2018;4(1):1-6.
25. Senthil kumar S. Studies on Grafting Techniques in Papaya (*Carica papaya* L.). M.sc. Thesis submitted to, Tamil Nadu Agricultural Univ., Coimbatore 2011.
26. Šeršeň F, Lácová M. Antioxidant activity of some coumarins Antioxidačná activita niektorých kumarínov. Acta Fac. Pharm. Univ. Comen 2015;LXII(9):41-45.
27. Singh L, Antil R, Dahiya P. Phytochemical screening and GC-MS analysis of methanolic extract of *Launaea procumbens* (Roxb.) Ramayya and Rajagopal. Annals of Biology 2020;36(3):436-441.
28. Uddin SJ, Bettadapura J, Guillon P, Darren Grice I, Mahalingam S, Tiralongo E. In-vitro antiviral activity of a novel phthalic acid ester derivative isolated from the Bangladeshi mangrove fern *Acrostichum aureum*. J Antivir Antiretrovir 2013;5(6):139-144.
29. Vankoten HW, Dlakic WM, Engel R, Cloninger MJ. Synthesis and biological activity of highly cationic dendrimer antibiotics. Molecular pharmaceutics 2016;13(11):3827-3834.
30. Zayed MZ, Wu A, Sallam SM. Comparative phytochemical constituents of *Leucaena leucocephala* (Lam.) leaves, fruits, stem barks, and wood branches grown in Egypt using GC-MS method coupled with multivariate statistical approaches. Bio Resources 2019;14(1):996-1013.
31. Zhao F, Wang P, Lucardi RD, Su Z, Li S. Natural sources and bioactivities of 2, 4-di-tert-butylphenol and its analogs. Toxins 2020;12(1):35.