

**Table S1. Representative Plant Metabolites Analyzed by Mass Spectrometry Imaging (Extended Table 1)**

Metabolite Class	Metabolites <sup>1</sup>	Plant Tissue	Reference	Method <sup>2</sup>	Spatial Resolution (μm)	MSI Instrument	MALDI Matrix(ces) <sup>3</sup>
Lipids	triacylglycerols (TAGs), phosphatidylcholines (PCs)	pennycress seed ( <i>Thlaspi arvense</i> L.)	(Johnston <i>et al.</i> , 2022) (Romsdahl <i>et al.</i> , 2021)	MALDI	40	Thermo Scientific MALDI-LTQ-Orbitrap-XL	DHB
	TAGs, PCs	rapeseed ( <i>Brassica napus</i> )	(Lu <i>et al.</i> , 2018)	MALDI	40	Thermo Scientific MALDI-LTQ-Orbitrap-XL	DHB
	PC- and TAG- cyclopropane fatty acids (FAs)	camelina seed ( <i>Camelina sativa</i> )	(Yu <i>et al.</i> , 2018)	MALDI	40	Thermo Scientific MALDI-LTQ-Orbitrap-XL	DHB
	TAGs, wax esters	jojoba seed ( <i>Simmondsia chinensis</i> )	(Sturtevant <i>et al.</i> , 2020)	MALDI	40	Thermo Scientific MALDI-LTQ-Orbitrap-XL	DHB
	TAGs with hydroxy FAs	castor seed ( <i>Ricinus communis</i> L.)	(Sturtevant <i>et al.</i> , 2019)	MALDI	40	Thermo Scientific MALDI-LTQ-Orbitrap-XL	DHB
	PCs	barrel medic nodule ( <i>Medicago truncatula</i> )	(Dokwal <i>et al.</i> , 2021)	MALDI	40	Thermo Scientific MALDI-LTQ-Orbitrap-XL	DHB
	PCs, lysophospholipids, phosphatidylglycerol (PG)	tomato leaf ( <i>Solanum lycopersicum</i> )	(Veličković <i>et al.</i> , 2021)	MALDI	20/75	Bruker Solarix 15T-FTICR and Bruker timsTOF flex	DHB, norharmane
	PCs, lysoPLs, phosphatidylethanolamine (PE), phosphatidic acid (PA), sulfoquinovosyl diacylglycerol (SQDG)	barley root ( <i>Hordeum vulgare</i> L.)	(Sarabia <i>et al.</i> , 2018)	MALDI	30	Bruker SolariX 7T XR hybrid ESI-MALDI-FT-ICR-MS	DHB
	PA, PE, PG, phosphatidylinositol (PI)	Arabidopsis leaf ( <i>Arabidopsis thaliana</i> )	(Mugume <i>et al.</i> , 2022)	MALDI	100	Thermo Scientific MALDI-LTQ-Orbitrap-Discovery	DHB, DAN
	oxylipins, FAs, ergosterol, glycerolipids	wheat grain ( <i>Triticum spp.</i> )	(Righetti <i>et al.</i> , 2022)	AP-MALDI	20	TransMIT AP-SMALDI5 AF-Thermo Fisher Scientific Q Exactive HF	DHB
	stigmastanol	carrot root ( <i>Daucus carota</i> )	(Xiang <i>et al.</i> , 2022)	AP-MALDI	20	Shimadzu MALDI-TOF-MS iMScope TRIO	DHB
Carbohydrates	sucrose and non-sucrose disaccharides	onion bulb ( <i>Allium cepa</i> )	(Zhan <i>et al.</i> , 2021)	MALDI	200	Bruker MALDI-TOF/TOF MS UltrafleXtreme	NEDC
	hexoses, sorbitol, sucrose	apple fruit ( <i>Malus domestica</i> )	(Horikawa <i>et al.</i> , 2019)	MALDI	500	Bruker MALDI-TOF/TOF MS UltrafleXtreme	DHB, CHCA
	hexoses, sucrose	strawberry fruit ( <i>Fragaria x ananassa</i> )	(Wang <i>et al.</i> , 2021) (Enomoto, 2021)	MALDI DESI	200 200	Bruker MALDI-TOF/TOF MS UltrafleXtreme Waters 2D DESI-Synapt XS Q-TOF-MS	DHB n/a
	(enzymatically degraded) cellulose, hemicellulose	maize stem ( <i>Zea mays</i> )	(Arnaud <i>et al.</i> , 2020)	MALDI	40	Bruker rapifleX MALDI Tissue typer MALDI-TOF-MS	DMA+DHB
	sucrose	grapevine leaf ( <i>Vitis vinifera</i> )	(Maia <i>et al.</i> , 2022)	MALDI	200	Bruker SolariX XR 9.4T hybrid ESI-MALDI-FT-ICR-MS	DHB, CHCA, 9-AA
Amino Acids	Cys, Asn, GABA, Gln, Lys	strawberry fruit ( <i>Fragaria x ananassa</i> )	(Nizioł <i>et al.</i> , 2019)	SALDI	100	Bruker Autoflex Speed TOF-MS	<sup>109</sup> Ag nanoparticles
	Ala, Asn, Gly, Gln Leu/Ile, Val	Maize root ( <i>Zea mays</i> )	(O'Neill and Lee, 2020)	MALDI	20	Thermo Scientific MALDI-LTQ-Orbitrap-Discovery	CA+gold
	Leu, Asn, Pro, His, Arg, Trp	lentil seedling ( <i>Lens culinaris</i> L.)	(Zhang <i>et al.</i> , 2023b)	AP-MALDI	15/45	Shimadzu MALDI-TOF-MS iMScope TRIO	CHCA
	spermidine, spermine	soybean seed ( <i>Glycine max</i> )	(Sagara <i>et al.</i> , 2020)	AP-MALDI	35	TransMIT AP-SMALDI10 AF-Thermo Fisher Scientific Q Exactive HF	DHB
	spermidine, spermine	Danshen root ( <i>Salvia miltiorrhiza</i> )	(Sun <i>et al.</i> , 2022)	MALDI	75	Bruker rapifleX MALDI Tissue typer MALDI-TOF-MS	DAN, BNDM

	<i>NI, N10</i> -diferuloylspermidine	pineapple fruit ( <i>Ananas comosus</i> )	(Suarez <i>et al.</i> , 2023)	MALDI	200	Bruker Autoflex Speed TOF-MS	CHCA, DHB
	aconitate, (iso)citrate, succinate, fumarate, malate	maize root ( <i>Zea mays</i> )	(Zhang <i>et al.</i> , 2023a)	DESI	80/130	Customized DESI probe with Thermo Scientific MALDI-LTQ-Orbitrap-XL	n/a
	malate, citrate	Arabidopsis seedling ( <i>Arabidopsis thaliana</i> ) liverwort seedling ( <i>Marchantia polymorpha</i> )	(Gomez-Zepeda <i>et al.</i> , 2021)	MALDI	50/200	Waters Synapt G1 HDMS	CHCA
Organic Acids	pyruvate, lactate, 2-ketobutyrate, maleate/fumarate, oxaloacetate, malate	strawberry fruit ( <i>Fragaria x ananassa</i> )	(Enomoto, 2021)	DESI	200	Waters 2D DESI-Synapt XS Q-TOF-MS	n/a
	malate, citrate, gluconate	notoginseng root ( <i>Panax notoginseng</i> )	(Sun <i>et al.</i> , 2021)	MALDI	100	Bruker rapifleX MALDI Tissue typer MALDI-TOF-MS	DHB, CHCA, 9-AA
	malate, maleate, citrate	Banlangen root ( <i>Isatis tinctoria</i> L.)	(Nie <i>et al.</i> , 2021)	AP-MALDI	80	Shimadzu MALDI-TOF-MS iMScope	DHAP, DAN
	malate, citrate	mango fruit ( <i>Mangifera indica</i> L.)	(Zhao <i>et al.</i> , 2022)	AFAI	100	AFAI-Thermo Fisher Scientific Q Exactive Plus	n/a
	liquiritigenin, apigenin, naringenin, luteolin, dihydrokaempferol, daidzein, quercetin, taxifolin, kaempferol, isorhamnetin, myricetin, catechin, quercetin 3- $\beta$ -d-glucoside, baicalin, rutin	Litchi seed ( <i>Litchi chinensis</i> Sonn.)	(Liu <i>et al.</i> , 2023b)	MALDI	250	Bruker Autoflex Speed TOF-MS	MBT
	quercetin, kaempferol, isorhamnetin	rapeseed stem ( <i>Brassica napus</i> )	(Krysa <i>et al.</i> , 2023)	MALDI	55	Waters Synapt G2-Si HDMS	CHCA
	caffeic acid, rosmarinic acid, other phenolic acids	Danshen stem, root, leaf, flower ( <i>Salvia miltiorrhiza</i> )	(Tong <i>et al.</i> , 2022)	DESI	100	Waters Xevo G2-XS QTOF	n/a
Phenolics	flavonoid aglycones, biflavonoids, flavonoid glycosides, biginkgosides	ginkgo leaf ( <i>Ginkgo biloba</i> )	(Li <i>et al.</i> , 2018)	MALDI	50	Bruker SolariX 7T XR hybrid ESI-MALDI-FT-ICR-MS	DHB, CHCA, 9-AA
	pinoresinol, phillygenin, forsythoside A, forsythoside E, rutin, caffeic acid	weeping Forsythia dried fruit ( <i>Forsythia suspensa</i> )	(Jing <i>et al.</i> , 2022)	MALDI	100	Bruker rapifleX MALDI Tissue typer MALDI-TOF-MS	DAN
	puerarin and derivatives, daidzin, mirificin, ambocin, hesperidin, ononin	<i>Puerariae</i> sp. dried root ( <i>Puerariae lobata</i> and <i>P. thomsonii</i> )	(Guo <i>et al.</i> , 2023)	AFA-DESI	200	Thermo Fisher Scientific Q Exactive	n/a
	nobiletin, tangeretin, tetramethoxyflavone, and feruloylputrescine, guaiacol	citrus leaf ( <i>Citrus sinensis-limonia</i> )	(de Moraes Pontes <i>et al.</i> , 2020)	DESI	200	Thermo Fisher Scientific Q Exactive	n/a
	apigenin, cannaflavin A, cannaflavin B, cannaflavin C, kaempferol, luteolin, orientin, quercetin, vitexin isovitexsin	cannabis leaf ( <i>Cannabis sativa</i> )	(Lorensen <i>et al.</i> , 2023b)	AP-MALDI	150/200	TransMIT AP-SMALDI5 AF-Thermo Fisher Scientific Q Exactive HF	DAN
	strictosidine, reserpine and derivatives, ajmalicine and derivatives, ajmaline and derivatives, serpentine	devil pepper root, stem, leaf, fruit ( <i>Rauvolfia tetraphylla</i> L.)	(Mohana Kumara <i>et al.</i> , 2019)	DESI	250	Prosolia Omni Spray-Thermo Scientific MALDI-LTQ-Orbitrap-XL	n/a
	reserpine and rescinnamine, and associated biosynthetic intermediates	devil pepper root, stem, leaf, fruit ( <i>Rauvolfia tetraphylla</i> L.)	(Lorensen <i>et al.</i> , 2023a)	AP-MALDI	15/20	TransMIT AP-SMALDI5 AF-Thermo Fisher Scientific Q Exactive HF	DHB
Alkaloids	atharanthine, vindolinine, serpentine, vindoline, anhydrovinblastine	periwinkle petal ( <i>Catharanthus roseus</i> )	(Dutkiewicz <i>et al.</i> , 2021)	SALDI	250	Bruker Autoflex Speed TOF-MS	TiO <sub>2</sub> nanowires
	gelsemine-, koumine-, gelsedine, humantenine-, yohimbine, sapargine-type alkaloids	heartbreak grass stem, root, leaf ( <i>Gelsemium legans</i> )	(Wu <i>et al.</i> , 2022b)	DESI	50	Waters Xevo G2-XS QTOF	n/a
	arecoline, arecaidine, caffeine, cotinine, guvacine, guvacoline, hordenine, sophoridine, trigonelline, vicine	Areca fruit ( <i>Areca catechu</i> )	(Wu <i>et al.</i> , 2022a)	MALDI	100	Bruker Autoflex Speed TOF-MS	DMCA

	(pseudo)ephedrine, methyl(pseudo)ephedrine	Ephedra shoot ( <i>Ephedra sinica</i> )	(Yun <i>et al.</i> , 2021)	DART	1000	IonSense DART- JEOL Accu-TOF-MS	n/a
	atharanthine, vindolinine, serpentine, vindoline and anhydrovinblastine	peyote crown and stem ( <i>Lophophora williamsii</i> )	(Lin <i>et al.</i> , 2023)	AP-MALDI	30	Shimadzu MALDI-TOF-MS iMScope QT	DTCB
	cocaine, truxilline, benzoylecgonine, cinnamoylcocaine	coca plant ( <i>Erythroxylum coca</i> )	(dos Santos <i>et al.</i> , 2021)	MALDI/LDI	150	Bruker SolariX XR 9.4T hybrid ESI-LDI/MALDI-FT-ICR-MS	CHCA, DHB, MBT
	(dehydro)tomatine and derivatives, (dehydro)esculeoside A	tomato fruit ( <i>Solanum lycopersicum</i> )	(Dong <i>et al.</i> , 2020; Kazachkova <i>et al.</i> , 2021)	MALDI	60/150	Bruker SolariX 7T XR hybrid ESI-MALDI-FT-ICR-MS	DHB
	lupanine, 13-hydroxylupanine, angustifoline	narrow-leafed lupin seed ( <i>Lupinus angustifolius</i> )	(Otterbach <i>et al.</i> , 2019)	MALDI	30	TransMIT AP-SMALDI10 AF-Thermo Fisher Scientific Q Exactive HF	DHB
	tomatine and derivatives, lycoperoside A-C and H	cherry tomato fruit ( <i>Lycopersicon esculentum</i> )	(Bednarz <i>et al.</i> , 2019)	MALDI	50	Bruker MALDI-TOF/TOF MS UltrafleXtreme	DHB
	solasodine and derivatives, chaconine	black nightshade fruit ( <i>Solanum nigrum</i> )	(Bednarz <i>et al.</i> , 2019)	MALDI	50	Bruker MALDI-TOF/TOF MS UltrafleXtreme	DHB
	tomatidenol, soladulcidine and derivatives	bittersweet nightshade fruit ( <i>Solanum dulcamara</i> )	(Bednarz <i>et al.</i> , 2019)	MALDI	50	Bruker MALDI-TOF/TOF MS UltrafleXtreme	DHB
Terpenes	vitexilactone, vitetrifolin B/E/F, rotundifuran	chaste tree fruit ( <i>Vitex agnus-castus</i> L.)	(Heskes <i>et al.</i> , 2018)	MALDI	40	Bruker SolariX 7T XR hybrid ESI-MALDI-FT-ICR-MS	DHB
	carosol and tanshinone plus pathway intermediates, other diterpenes	<i>S. grandifolia</i> and Danshen root ( <i>Salvia grandifolia</i> and <i>S. miltiorrhiza</i> )	(Zhang <i>et al.</i> , 2023b)	AP-MALDI	15/45	Shimadzu MALDI-TOF-MS iMScope TRIO	CHCA
	kaurane diterpenes, xylopic acid	Ethiopian pepper fruit ( <i>Xylopiya aethiopica</i> )	(Kyekyeku <i>et al.</i> , 2020)	MALDI	15	Thermo Scientific MALDI-LTQ-Orbitrap-XL	CHCA
	platycodin D, platycodin D3 platycoside E	balloon flower root ( <i>Platycodon grandiflorum</i> )	(Tang <i>et al.</i> , 2023)	MALDI	50	Bruker timsTOF fleX	DHB
	carosol, kahweol, lactaroviolin, squalene, toxoids	Taxus leaf ( <i>Taxus mairei</i> )	(Zhan <i>et al.</i> , 2023)	MALDI	20	Bruker timsTOF flex MALDI-2	DHB+DHAP
	momilactone-A/B, phytocassane-A-E	Thai rice leaf ( <i>Oryza sativa</i> )	(Komkleow <i>et al.</i> , 2021)	MALDI	50	MALDI-SpiralTOF (JMS-S3000)	Fe <sub>3</sub> O <sub>4</sub>
	soyasaponins	soybean root nodules ( <i>Glycine max</i> )	(Agtuca <i>et al.</i> , 2020)	LAESI	200	Custom LAESI-Waters Synapt G2S	n/a
	nobilomethylene, dendronobilin F/K, rupestonic acid G, isopetasol, dendroside G, dendroterpene C	noble dendrobium stem ( <i>Dendrobium nobile</i> )	(Liu <i>et al.</i> , 2023a)	MALDI	20	Bruker AutoFlex Speed MALDI TOF	MBT
Vitamins and Pigments	(Vit E) tocopherols, tocotrienols	upland cotton seed ( <i>Gossypium hirsutum</i> )	(Salimath <i>et al.</i> , 2021)	MALDI	80	Thermo Scientific MALDI-LTQ-Orbitrap-XL	DHB
	Vit A1, Vit B1, Vit B6, Vit C	raw and dried persimmon fruit ( <i>Diospyros kaki</i> )	(Shikano <i>et al.</i> , 2020)	MALDI	120	Bruker rapifleX MALDI Tissuetyper MALDI-TOF-MS	CHCA
	7-dehydrocholesterol (provitamin D3), cholesterol	tomato fruit ( <i>Solanum lycopersicum</i> )	(Li <i>et al.</i> , 2022)	MALDI	105	Waters Synapt G2-Si HDMS	DHB
	anthocyanins	strawberry fruit ( <i>Fragaria x ananassa</i> )	(Wang <i>et al.</i> , 2021)	MALDI	200	Bruker MALDI-TOF/TOF MS UltrafleXtreme	DHB
	betalains, chlorophyll a	tobacco leaf ( <i>Nicotiana benthamiana</i> )	(Dong <i>et al.</i> , 2020)	MALDI	60	Bruker SolariX 7T XR hybrid ESI-MALDI-FT-ICR-MS	DHB
	β-carotene	carrot root ( <i>Daucus carota</i> )	(Xiang <i>et al.</i> , 2022)	AP-MALDI	20	Shimadzu MALDI-TOF-MS iMScope TRIO	DHB
	polyacetylenes	Cangzhu root ( <i>Atractylodes lancea</i> )	(Jiang <i>et al.</i> , 2022)	DESI/PI	200	Agilent 6224 Accurate-Mass TOF	n/a
Cannabinoids	Δ9-THCA, CBNA	cannabis leaf ( <i>Cannabis sativa</i> )	(dos Santos <i>et al.</i> , 2019)	MALDI/LDI	150	Bruker SolariX XR 9.4T hybrid ESI-LDI/MALDI-FT-ICR-MS	CHCA, DHB, MBT
	Δ9-THCA, CBN(A), CBE(A), CBGA	cannabis leaf ( <i>Cannabis sativa</i> )	(Lorensen <i>et al.</i> , 2023b)	AP-MALDI	150/200	TransMIT AP-SMALDI5 AF-Thermo Fisher Scientific Q Exactive HF	DAN

Glucosinolates	indol-3-ylmethyl glucosinolate (GSL), 4-methylthiobutyl GSL, methylthiooctyl GSL, and other GSLs	arabidopsis leaf ( <i>Arabidopsis thaliana</i> )	(Morikawa-Ichinose <i>et al.</i> , 2020)	MALDI	50	Shimadzu AXIMA Confidence MALDI-TOF	9-AA
	isatindigoside F GSL and other GSLs	Banlangen root ( <i>Isatis tinctoria</i> L.)	(Nie <i>et al.</i> , 2021)	DESI	80	Waters 2D DESI-Synapt XS Q-TOF-MS	n/a
Hormones	brassinosteroid, salicylic acid, 1-aminocyclopropane-1-carboxylic acid, abscisic acid, cytokinin auxin	rice root ( <i>Oryza sativa</i> )	(Shiono and Taira, 2020)	Nano-PALDI	15	Bruker MALDI-TOF/TOF MS UltrafleXtreme	Fe-nanoparticles
Nucleotide bases	guanine, adenine, adenosine	Banlangen root ( <i>Isatis tinctoria</i> L.)	(Nie <i>et al.</i> , 2021; Nie <i>et al.</i> , 2022)	DESI AP-MALDI	80 80	Waters 2D DESI-Synapt XS Q-TOF-MS Shimadzu MALDI-TOF-MS iMScope	n/a DHAP, DAN

<sup>1</sup>Not all metabolites are listed for each study. Studies are generally categorized by predominant and/or unique metabolites imaged.

<sup>2</sup>Techniques: matrix-assisted laser desorption/ionization (MALDI), atmospheric pressure-MALDI (AP-MALDI), surface-assisted laser desorption/ionization (SALDI), desorption electrospray ionization (DESI), air flow-assisted ionization (AFAI), direct analysis in real-time (DART), DESI with post-photoionization assembly (DESI/PI)

<sup>3</sup>Matrices: 2,5-dihydroxybenzoic acid (DHB),  $\beta$ -Carboline (norharmane), 1,5-diaminonaphthalene (DAN), N-(1-naphthyl) ethylenediamine dihydrochloride (NEDC), alpha-cyano-4-hydroxycinnamic acid (CHCA), N,N-dimethylaniline (DMA), 9-aminoacridine (9-AA), 4-hydroxy-3-cinnamaldehyde (CA), 1,1'-binaphthyl-2,2'-diamine (BNDM), 2', 5'-dihydroxyacetophenone (DHAP), trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DTCB), 2-mercaptobenzothiazole (MBT), 3,4-dimethoxycinnamic acid (DMCA)

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