Supplementary Information

Metabolomics-Driven Identification of Resistance Biomarkers in Dwarf Cashew (*Anacardium occidentale* L.) under Black Mold Stress

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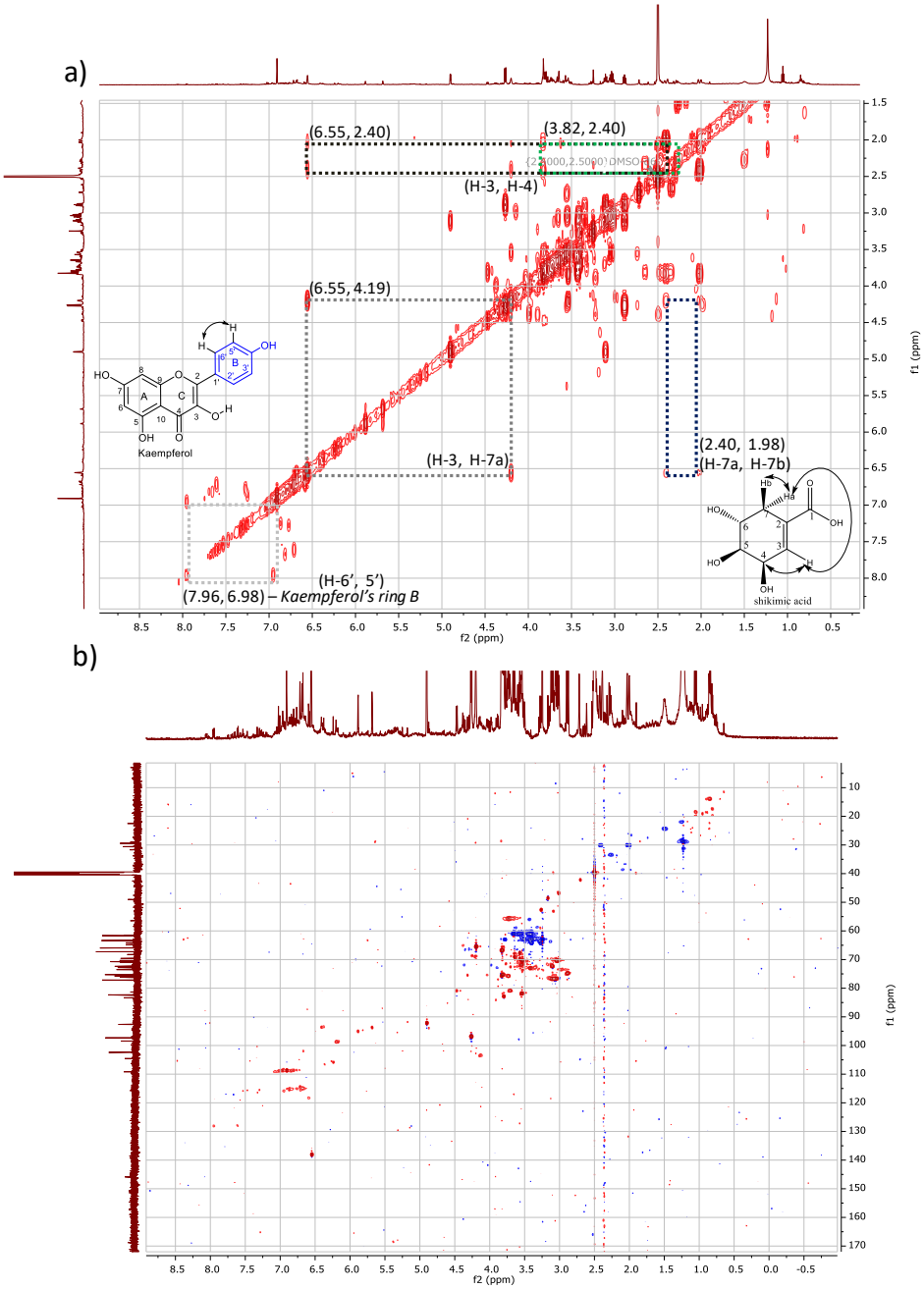


Figure S1. 2D NMR of cashew leaves extracts in DMSO-d_6 (26 °C, AVANCE 600 MHz): (a) COSY contour map. (b) Multiplicity-edited HSQC contour map color-coded to display different carbon-proton groups: red = –C**H**–/–C**H**$_3$ and blue = –C**H**$_2$– groups.

Table S1. ^{1}H (600 MHz) and ^{13}C chemical shifts (150 MHz), coupling constants, multiplicity, and long-range heteronuclear ^{1}H-^{13}C HMBC correlations for the 18 cashew clone's metabolites (25 °C, DMSO-d_6)

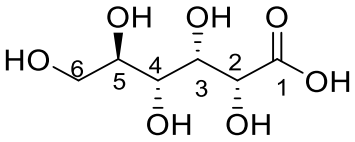
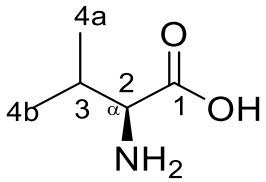
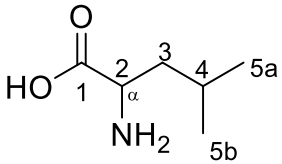
FooDB ID[g]	*m/z* [M – H]$^{-}$	Name and numbered structure[a-d]	δ ^{1}H / ppm, (multiplicity, *J* / Hz)	^{13}C / ppm HSQC H→C	C-n HMBC H→C	Reference
FDB001980	195.0505	gluconic acid (**1**) 	δ 4.21 (d, 2.6, 1H-2)[d]	nd (C-1)		
			δ 4.09 (m, o, 1H-3)[d]	76.3 (C-2)		1
			δ 3.55 (m, o, 1H-4)	73.1 (C-3)	C-2	
			δ 3.74 (m, o, 1H-5)	73.6 (C-4)		
			δ 3.64 (m, o, 1H-6a)[d]	74.1 (C-5)		
			δ 3.79 (m, o, 1H-6b)[d]	65.2 (C-6)		
FDB004905	116.1512	valine 	δ 3.68 (m, o, 1H-2)	nd (C-1)		
			δ 2.27 (m, o, 1H-3)	62.4 (C-2)		
			δ 0.95 (d, 6.8, 3H-4a)[d]	31.9 (C-3)	C-3	2
			δ 0.98 (d, 6.8, 3H-4b)[d]	19.0 (C-4)		
FDB000899	116.1512	leucine 	δ 3.85 (m,1H-2)	nd (C-1)		
			δ 1.77 (m,2H-3)	56.4 (C-2)		3,4
			δ 1.80 (m, 1H-4)	41.9 (C-3)		
			δ1.03 (t, 7.3, 1H-5a)	25.0 (C-4)		
			δ 0.99 (m, 3H-5b)	24.4 (C-5)		

Table S1. ^{1}H (600 MHz) and ^{13}C chemical shifts (150 MHz), coupling constants, multiplicity, and long-range heteronuclear ^{1}H-^{13}C HMBC correlations for the 18 cashew clone's metabolites (25 °C, DMSO-d_6) (cont.)

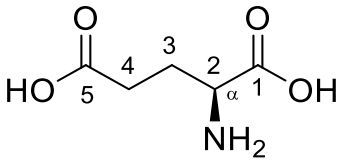
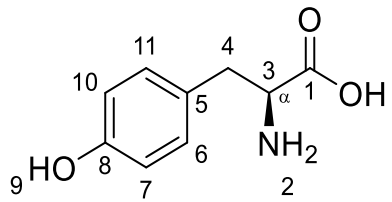
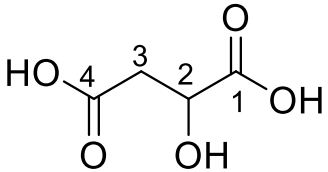
FooDB ID[g]	*m/z* [M – H]⁻	Name and numbered structure [a-d]	δ ^{1}H / ppm, (multiplicity, *J* /Hz)	$\delta^{13}C$ / ppm HSQC H→C	C-n HMBC H→C	Reference
FDB012535	146.1301	glutamic acid 	δ 3.83 (m, 1H-2)[d]	185.5 (C-1)		5,6
			δ 2.00 (d, 4.1, 2H-3a)[d]	59.9 (C-2)		
			δ 2.03 (d, 4.1, 2H-3b)[d]	30.6 (C-3)		
			δ 2.40 (m, 2H-4)[d]	35.8 (C-4)		
FDB000446	180.1900	tyrosine 	δ 3.90 (d, 5.9, 1H-3)	59.9 (C-3)		7
			δ 7.03 (m, 1H-2)[d]	69.0 (C-2)		
			δ 6.99 (m, 2H-3a)[d]	39.1 (C-3)		
FDB008114	133.0129	malic acid (**3**) 		nd (C-1 e C-4)		
			27 (m, 1H-2)[d]	69.0 (C-2)		
			δ 2.88 (m, 2H-3a)[d]	39.1 (C-3)		3

Table S1. ^{1}H (600 MHz) and ^{13}C chemical shifts (150 MHz), coupling constants, multiplicity, and long-range heteronuclear ^{1}H-^{13}C HMBC correlations for the 18 cashew clone's metabolites (25 °C, DMSO-d_6) (cont.)

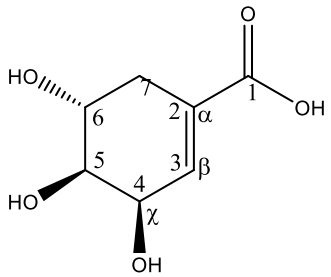
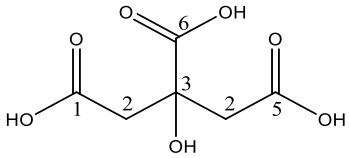
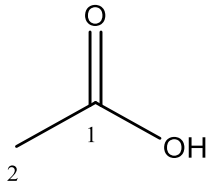
FooDB ID[g]	*m/z* [M – H]$^{-}$	Name and numbered structure [a-d]	δ ^{1}H / ppm, (multiplicity, *J* /Hz)	$\delta^{13}C$ / ppm HSQC H→C	C-n HMBC H→C	Reference
FDB003991	173.0441	shikimic acid (**3**)	δ 6.55 (m, o, 1H-3)[d]	168.3 (C-1)		
			δ 4.19 (bs, 1H-4)[d]	136.2 (C-2)		
			δ 3.77 (m, o, 1H-5)	138.3 (C-3)		
			δ 3.79 (m, o, 1H-6)[d]	69.4 (C-4)		
			δ 2.40 (m, 1H-7a)[d]	66.9 (C-5)		2,8
			δ 1.98 (m, 1H-7b)[d]	67.8 (C-6) 30.3 (C-7)		
FDB012586	191.0183	citric acid (**5**)		nd (C-1,5,6)		
			δ 2.72 (m, o, 2H-2a)	48.9 (C-2)		3
			δ 2.53 (m, o, 2H-2b)	77.8 (C-3)		
FDB008299	59.0000	acetic acid				
			δ 1.90 (s, 3H-2)	178.1 (C-1) 24.7 (C-2)	C-1	

Table S1. ^{1}H (600 MHz) and ^{13}C chemical shifts (150 MHz), coupling constants, multiplicity, and long-range heteronuclear ^{1}H-^{13}C HMBC correlations for the 18 cashew clone's metabolites (25 °C, DMSO-d_6) (cont.)

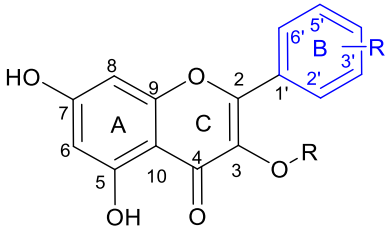
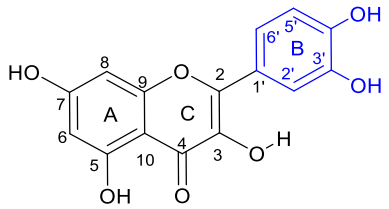
FooDB ID[g]	*m/z* [M – H]$^{-}$	Name and numbered structure[a-d]	δ ^{1}H / ppm, (multiplicity, *J* /Hz)	$\delta^{13}C$ / ppm HSQC H→C	C-n HMBC H→C	Reference
		flavonol skeleton	ring A			
			δ 6.19 (m, o, 1H-6)	163.0 (C-5)		
			δ 6.40 (m, o, 1H-8)	97.3 (C-6)		
				166.8 (C-7)		
			Ring C			
			-	145.8 (C-2)		
			-	173.9 (C-4)		
FDB011904	301.0328	quercetin (**24**)	Ring B			
			δ 6.67 (m, o, 1H-5')	126.1 (C-1')		
				115.9 (C-2')		
			δ 6.94 (m, o, 1H-5')	145.3 (C-3')		9
			δ 7.68 (d, 2.0, 1H-6')	146.5 (C-4')		
				116.1 (C-5')		
				126.1 (C-6')		

Table S1. ^{1}H (600 MHz) and ^{13}C chemical shifts (150 MHz), coupling constants, multiplicity, and long-range heteronuclear ^{1}H-^{13}C HMBC correlations for the 18 cashew clone's metabolites (25 °C, DMSO-d_6) (cont.)

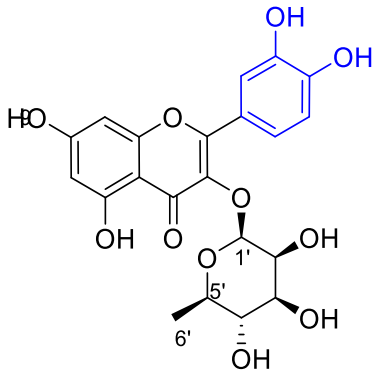
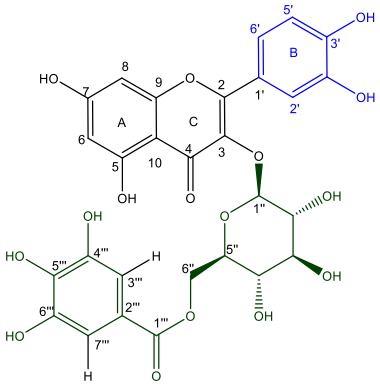
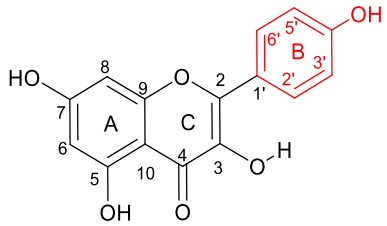
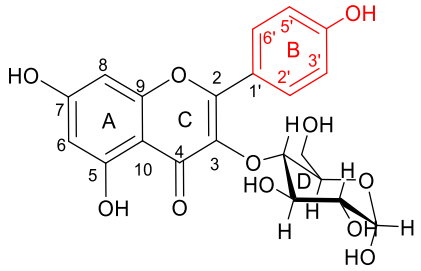
FooDB ID[g]	*m/z* [M – H]$^{-}$	Name and numbered structure [a-d]	δ ^{1}H / ppm, (multiplicity, *J* / Hz)	δ ^{13}C / ppm HSQC	C-n HMBC H→C	Reference
FDB011884	447.0898	quercetin-3-*O*-rhamnoside (**30**)	rhamnosyl unit			
			δ 5.18 (d, 3.8, 1H-1'')[d]	102.1 (C-1'')		
			δ 3.81 (m, o, 2H-2")[d]	76.1 (C-2'')		10
			δ 3.75 (m, o, 2H-3")	73.2 (C-3'')		
			δ 3.78 (m, o, 2H-4'')	73.0 (C-4'')		
			δ 3.73 (m, o, 2H-5'')	75.3 (C-5'')		
	615.0959	quercetin-3-*O*-(6-*O*-galloyl)-glucoside (**25**)	hexosyl unit			
			δ 5.25 (d, 3.7, 1H-1''')	104.1 (C-1'')		
			δ 3.81 (dd, 3.5 and 1.5, 2H-2''')	75.1 (C-2'')		
			δ 3.75 (m, 2H-3''')	72.2 (C-3'')		11
			δ 3.71 (m, 2H-4''')	73.3 (C-4'')		
			δ 3.70 (m, 2H-5''')	74.1 (C-5'')		
			δ 4.31 (m, 1H-6a''')	75.2 (C-6'')		
			δ 3.76 (m, 1H-6b''')			

Table S1. ^{1}H (600 MHz) and ^{13}C chemical shifts (150 MHz), coupling constants, multiplicity, and long-range heteronuclear ^{1}H-^{13}C HMBC correlations for the 18 cashew clone's metabolites (25 °C, DMSO-d_6) (cont.)

FooDB ID[g]	*m/z* [M – H]$^{-}$	Name and numbered structure[a-d]	δ ^{1}H / ppm, (multiplicity, *J* /Hz)	δ ^{13}C / ppm HSQC	C-n HMBC H→C	Reference
			galloyl unit δ 6.90 (s, 1H-3''' and 7''')	167.9 (C-1''') 120.3 (C-2''') 108.3 (C-3''' and 7''') 146.5(C-4'''and C6''') 140.2 (C-5''')		
FDB000633	285.0399	kaempferol (**29**) 	Ring B δ 7.96 (d, 8.8, 2H-2',6')[d]	123.3 (C-1') 128.3 (C-2',6') 156.1 (C-4') 115.4 (C-3',5')		
FDB004135	447.0990	kaempferol-3-*O*-hexoside 	Hexosyl unit – Ring D δ 4.90 (d, 3.5, 1H-1'') δ 3.55 (m, 2H-2'') δ 3.51 (m, 2H-3'') δ 3.43 (m, 2H-4'') δ 3.55 (m, 2H-5'')	92.6 (C-1") 75.6 (C-2") 77.6 (C-3") 72.6 (C-4") 77.6 (C-5")		12

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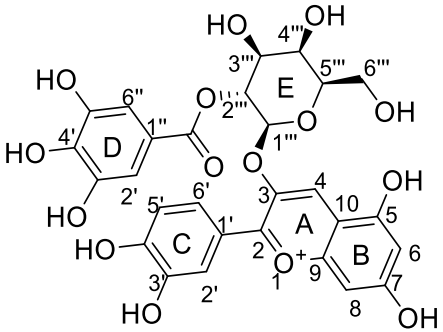
FooDB ID[g]	*m/z* [M – H]⁻	Name and numbered structure[a-d]	δ ^{1}H / ppm, (multiplicity, *J* / Hz)	δ ^{13}C / ppm HSQC	C-n HMBC H→C	Reference
	599.1037	cyanidin-3-*O*-(2''galloyl)-galactoside (**34**)	δ 3.69 (m, 2H-6'')	69.6 (C-6")		
			Ring A	167.1 (C-2)		
			δ 6.55 (s, o, H-4)	145.8 (C-3)	C-10, C-3	
				138.0 (C-4)		13,14
			Ring B	170.3 (C-7)		
			δ 6.23 (m, o, H-6)	98.0 (C-8)		
			δ 6.19 (m, o, 2.0, H-8)	157.8 (C-9)		
				114.2 (C-10)		
			Ring C	123.1 (C-1')		
			δ 7.61 (m, o, H-2')	118.1 (C-2')		
			δ 7.66 (8.7 Hz, H-5')	146.4 (C-3')		
			δ 7.61 (m, o, H-2')	145.1 (C-4')		
			Galloyl unit - Ring D	128.3 (C-5')	C-4',C-5'	
			δ 6.67 (s, 1H-2'' and 6'')	116.3 (C-6')		
			Galloyl unit - Ring D			
			δ 6.67 (s, 1H-2'' and 6'')	167.1 (C-1")		

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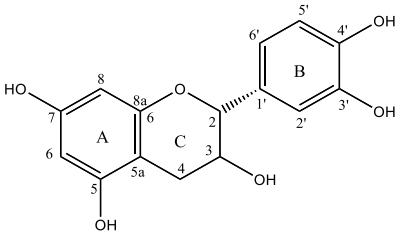
FooDB ID[g]	*m/z* [M – H]$^-$	Name and numbered structure[a-d]	δ ^{1}H / ppm, (multiplicity, *J* / Hz)	δ ^{13}C / ppm HSQC	C-n HMBC H→C	Reference
				108.7 (C-2"and 6")		
				146.5 (C-3" and C-5")		
			hexosyl unit - ring E	140.2 (C-4")		
			δ 6.19 (d, 3.5, 1H-1"')	99.0 (C-1"')		
			δ 4.90 (d, 3.5, 1H-2"')	74.4 (C-2"')		
			δ 3.63 (m, 2H-3"')	73.2 (C-3"')		
			δ 3.61 (m, 2H-4"')	71.3 (C-4"')		
			δ 3.57 (m, o, 2H-5"')	79.5 (C-5"')		
			δ 3.53 (m, o. 2H-6'")	69.5 (C-6"')		
FDB002571	289.0716	cathechin (**14**)				
			Ring C			
			δ 4.81 (br s, H-2)	90.1 (C-2)		
			δ 4.86 (d, 3.6 Hz, H-3)	65.6 (C-3)		
			δ 2.82 (d, 3.6 Hz, H-4)	28.4 (C-4)		15
			Ring A			
				159.0 (C-5)		

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FooDB ID[g]	*m/z* [M – H]$^{-}$	Name and numbered structure[a-d]	δ ^{1}H / ppm, (multiplicity, *J* / Hz)	δ ^{13}C / ppm HSQC H→C	C-n HMBC H→C	Reference
			δ 5.88 (d, 2.4 Hz, H-6)	95.0 (C-6)		
				158.4 (C-7)		
			δ 5.68 (d, 2.4 Hz, H-8)	95.6 (C-8)		
				157.3 (C-8a)		
				101.0 (C-5a)		
			Ring B			
				129.6 (C-1')		
			δ 6.80 (d, 1.6, 1H-2')	115.0 (C-2')		
				146.8 (C-3')		
				145.3 (C-4')		
			δ 6.75 (d, 8.0, 1H-5')	116.8 (C-5')		
			δ 6.70 (d, 8.0, 1.6, 1H-6')	121.3 (C-6')		

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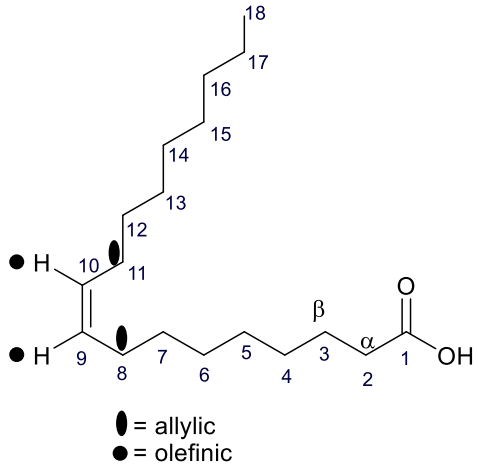
FooDB ID[g]	*m/z* [M – H]$^-$	Name and numbered structure[a-d]	δ ^{1}H / ppm, (multiplicity, *J* / Hz)	δ ^{13}C / ppm HSQC H→C	C-n HMBC H→C	Reference
FDB012858	281.2495	oleic acid				
			δ 2.27 (m, 2H-2)[d]	34.2 (C-2)		
			δ 1.94 (m, o, 2H-3)[d]	27.9 (C-3)		
			δ 1.47 (m, o, 2H-4)[d]	25.4 (C-4)		16,17
			δ 1.29 (m, o, 2H-5)	29.9 (C-5)		
			$\underline{\delta}$1.28 (m, o, 2H-6)	29.9 (C-6)		
			δ 1.30(m, o, 2H-7)	29.9 (C-7)		
			δ 1.94 (m, o, 2H-8,11)	27.1 (C-8,11)		
			δ 5.29 (m, o, 2H-9,10)	130.2 (C-9,10)		
			δ 1.29 (m, o, 2H-12)	31.6 (C-12)		
			δ 1.30 (m, o, 2H-13)	28.9 (C-13)		
			δ 1.33 (m, o, 2H-14)	28.5 (C-14)		
			δ 1.26 (m, o, 2H-15)	29.3 (C-15)		
			δ 1.23 (m, 2H-16)	22.7 (C-16)		
			δ 0.79 (t, 6.8, 3H-18)	14.9 (C-17)		

[a]Multiplicity types: s (singlet), broad singlet (bs), d (doublet), t (triplet), q (quartet), h (heptet), m (multiplet); [b]Notes: (o) for overlapping, (nd) for non-detectable signal. [c]All chemical shifts (δ) were assigned to carbon-13 (^{13}C) and (^{1}H) proton data using (^{1}H-^{13}C)-HSQC multiplicity editing and (^{1}H-^{13}C) HMBC experiments. [d]For some sets of (^{1}H) protons the assignment was performed using (^{1}H-^{1}H)-COSY experiments. [e]The chemical shift used in the metabolomic study is highlighted in bold. [f]The spectroscopic data of amino acids, catechin and quercetin were compared with analytical standards. [g]FooDB ID code obtained from reference 18. The number in parentheses-reported in the Name and numbered structure column-corresponds to the annotation number for that substance in Table 2 (mass spectrometry data, in the article).

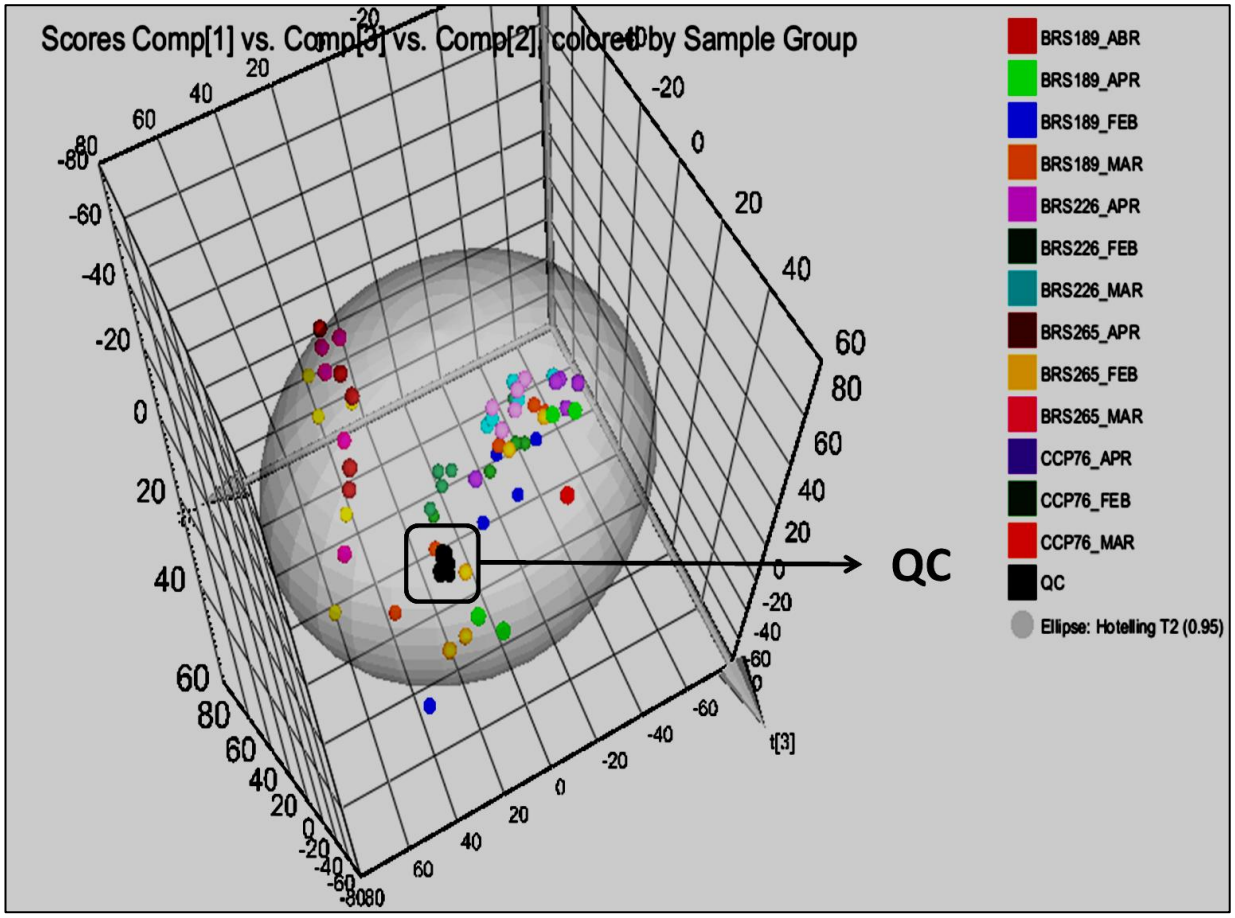


Figure S2. Three-dimensional PCA score plot (PC1 *vs.* PC2 *vs.* PC3) displaying the clustering of cashew genotypes based on metabolomic profiles associated with resistance to black mold (*P. anacardii*). Genotypes BRS 226 (intermediary resistance), BRS 265 (resistant), and susceptible genotypes CCP 76 and BRS 189 are distinctly grouped. Score chart comparing the quality control samples (QCs) with the samples of dwarf cashew genotypes. Hotelling's T^2 ellipse (95% confidence interval).

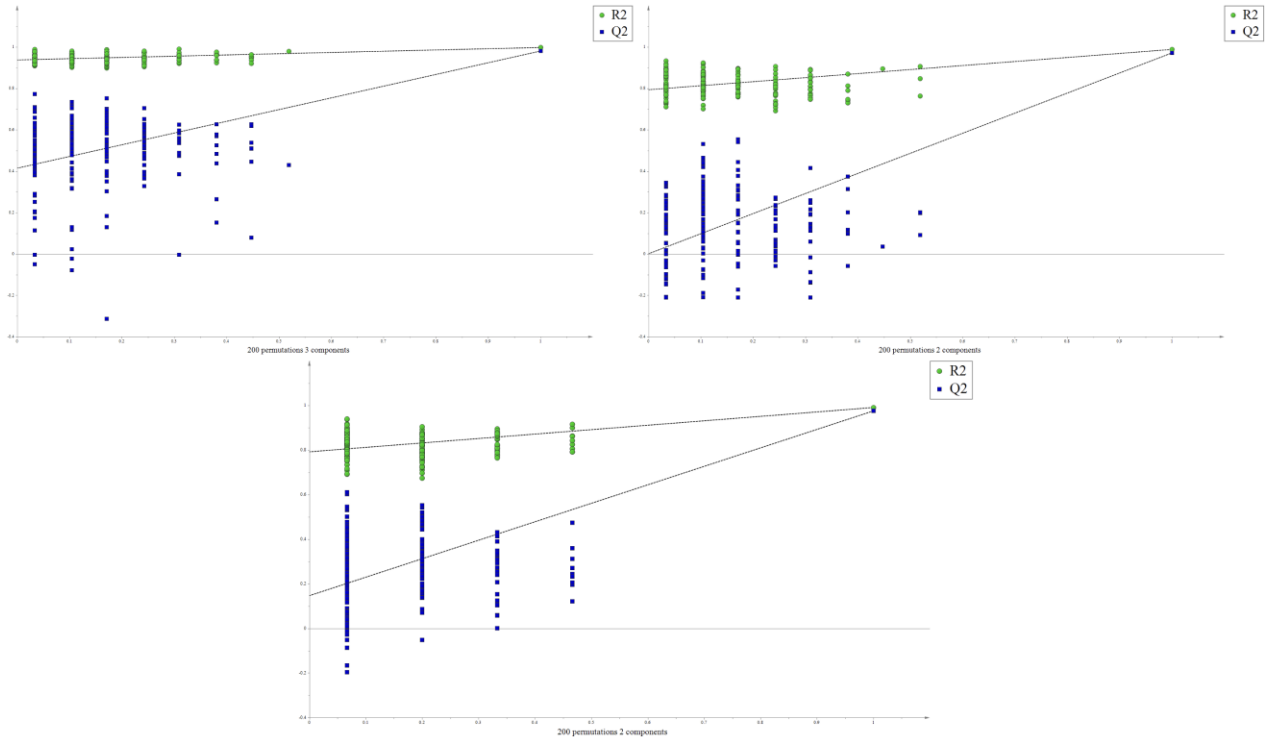


Figure S3. Permutation tests for the OPLS-DA models presented in Figure 4, validating the discrimination of resistant (BRS 265), susceptible 1 (CCP 76), susceptible 2 (BRS 189), and intermediate (BRS 226) dwarf cashew genotypes under black mold stress.

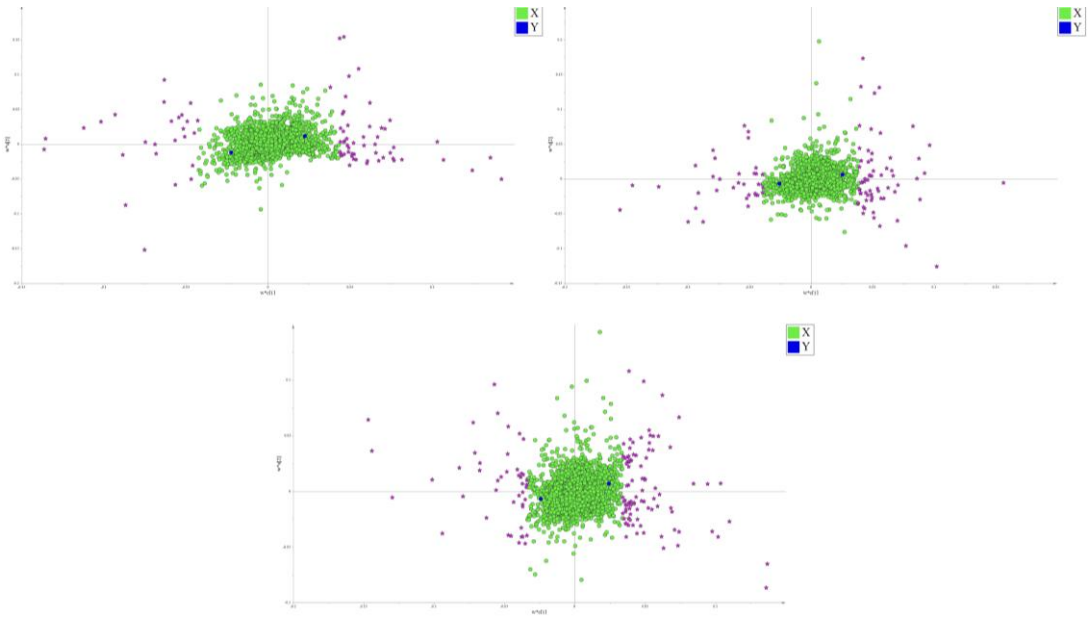


Figure S4. Variable Importance in Projection (VIP) scores and Loading Plot from the OPLS-DA models shown in Figure 4, highlighting the metabolites contributing to the discrimination of resistant (BRS 265), susceptible 1 (CCP 76), susceptible 2 (BRS 189), and intermediate (BRS 226) dwarf cashew genotypes under black mold stress.

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