

Supplementary Material

Molecular Authentication and Phytochemical Evaluation of Indigenous Germplasm of Genus *Physalis* for Sustainable Utilization

Katherine Pere ¹, Kenneth Mburu ², Edward K. Muge ¹, John Maina Wagacha ³ and Evans N. Nyaboga ^{1,*}

¹ Department of Biochemistry, University of Nairobi, P.O. Box 30197, Nairobi 00100, Kenya; perekatherine@gmail.com (K.P.); mugeek@uonbi.ac.ke (E.K.M.)

² Department of Life Sciences, South Eastern Kenya University, P.O. Box 170, Kitui 90200, Kenya; kmburu@seku.ac.ke

³ Department of Biology, University of Nairobi, P.O. Box 30197, Nairobi 00100, Kenya; maina.wagacha@uonbi.ac.ke

* Correspondence: nyaboga@uonbi.ac.ke

Supplementary Tables

Supplementary Table S1: BLASTn analysis results for *Physalis* accessions based on ITS2 and *rbcL* barcode regions

	<i>rbcL</i>					ITS2				
Sample ID	Species of Best BLAST match	GenBank accession number (of database)	E value	Percent identity (%)	GenBank accession number	Species of Best BLAST match	GenBank accession number (of database)	E value	Percent identity (%)	GenBank accession number
L1	<i>Physalis minima</i>	NC_048515.1	0.0	100	OQ507152.1	-	-	-	-	-
L2	<i>Physalis minima</i>	NC_048515.1	0.0	100	OQ507153.1	<i>Physalis purpurea</i>	MH763740.1	0.0	92.98	OQ372021.1
L3	<i>Physalis minima</i>	NC_048515.1	0.0	99.59	OQ507154.1	<i>Physalis purpurea</i>	MH763740.1	0.0	94.06	OQ372022.1
L4	<i>Physalis minima</i>	NC_048515.1	0.0	100	OQ507155.1	<i>Physalis peruviana</i>	AY665914.1	0.0	97.41	OQ372023.1
L5	<i>Physalis minima</i>	NC_048515.1	0.0	99.86	OQ507156.1	<i>Physalis purpurea</i>	MH763740.1	0.0	94.45	OQ372024.1
L6	<i>Physalis minima</i>	NC_048515.1	0.0	100	OQ507157.1	<i>Physalis aff. philadelphica</i>	AY665868.1	2e-144	91.35	OQ372025.1
L7	<i>Physalis minima</i>	NC_048515.1	0.0	100	OQ507158.1	<i>Physalis purpurea</i>	MH763740.1	1e-148	86.00	OQ372026.1
L8	<i>Physalis minima</i>	NC_048515.1	0.0	100	OQ507159.1	<i>Physalis purpurea</i>	MH763740.1	0.0	93.07	OQ372027.1
L9	<i>Physalis minima</i>	NC_048515.1	0.0	100	OQ507160.1	<i>Physalis purpurea</i>	MH763740.1	0.0	88.96	OQ372028.1
L10	<i>Physalis</i>	NC_048515.1	0.0	100	OQ507161.1	<i>Physalis</i>	MH763740.1	0.0	90.16	OQ372029.1

	<i>minima</i>					<i>purpurea</i>	1			
--	---------------	--	--	--	--	-----------------	---	--	--	--

Supplementary Figures

Supplementary Figure S1: Multiple sequence alignment for *Physalis* accessions ITS2 and *rbcL* gene sequences as well as their reference sequences based on BLASTn analysis (<https://espript.ibcp.fr/ESPrIPT/temp/1818399141/0-0-1680464456-esp.pdf>)

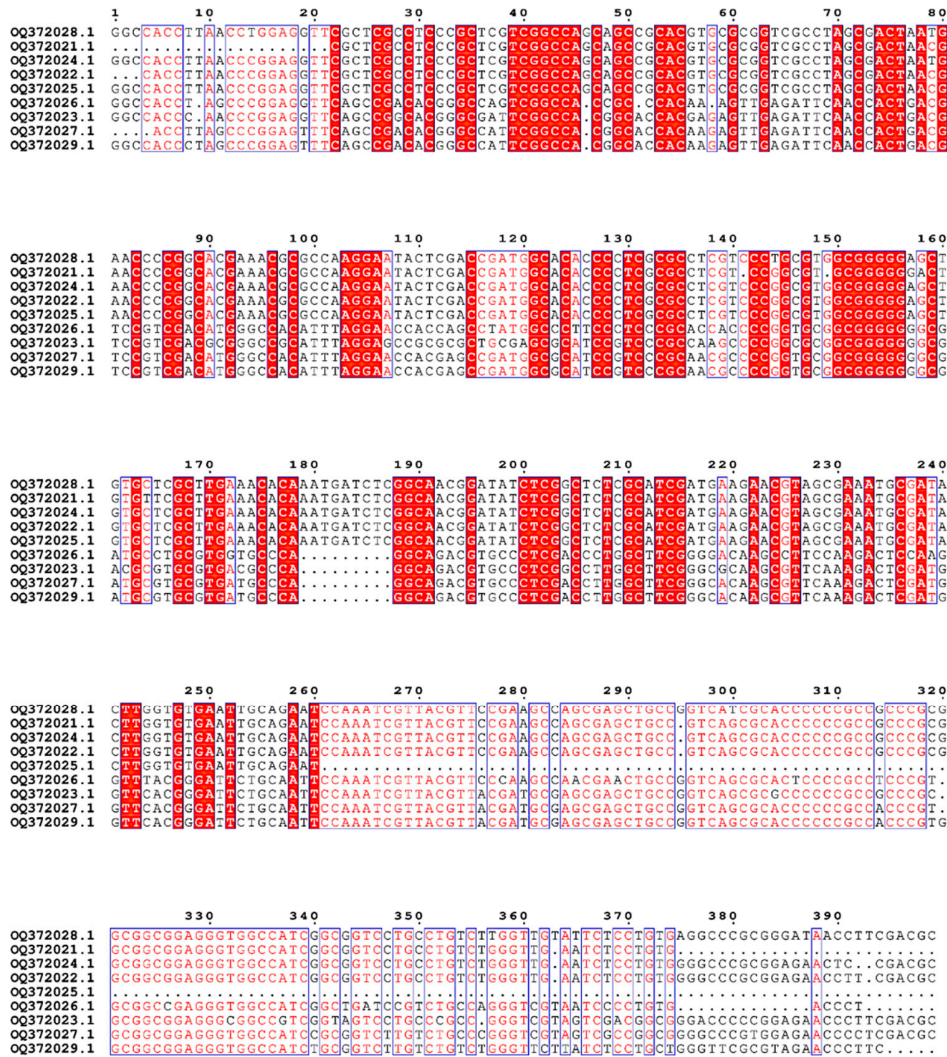
```
1      10      20      30      40      50      60      70
KT178121.1* ATGTCACCAAAACAGAGACTAAAGCAAGTGTGGATTCAAGGCTGGTGTAAAGAGTACAAATTGACTTATTACTC
NC_026570.1* ATGTCACCAAAACAGAGACTAAAGCAAGTGTGGATTCAAGGCTGGTGTAAAGAGTACAAATTGACTTATTACTC
KT178120.1* ATGTCACCAAAACAGAGACTAAAGCAAGTGTGGATTCAAGGCTGGTGTAAAGAGTACAAATTGACTTATTACTC
NC_048515.1* ATGTCACCAAAACAGAGACTAAAGCAAGTGTGGATTCAAGGCTGGTGTAAAGAGTACAAATTGACTTATTACTC
NC_048514.1* ATGTCACCAAAACAGAGACTAAAGCAAGTGTGGATTCAAGGCTGGTGTAAAGAGTACAAATTGACTTATTACTC
NC_070364.1* ATGTCACCAAAACAGAGACTAAAGCAAGTGTGGATTCAAGGCTGGTGTAAAGAGTACAAATTGACTTATTACTC
NC_039458.1* ATGTCACCAAAACAGAGACTAAAGCAAGTGTGGATTCAAGGCTGGTGTAAAGAGTACAAATTGACTTATTACTC
NC_039457.1* ATGTCACCAAAACAGAGACTAAAGCAAGTGTGGATTCAAGGCTGGTGTAAAGAGTACAAATTGACTTATTACTC
OQ507152.1 .....
OQ507153.1 .....
OQ507154.1 .....
OQ507155.1 .....
OQ507156.1 .....
OQ507157.1 .....
OQ507158.1 .....
OQ507159.1 .....
OQ507160.1 .....
OQ507161.1 .....
AY665903.1* .....
AY665868.1* .....
AY665910.1* .....
AY665914.1* .....
AY665879.1* .....
AY665905.1* .....
AY665886.1* .....
MH763728.1* .....
MH763740.1* .....
OQ372021.1 .....
OQ372022.1 .....
OQ372023.1 .....
OQ372024.1 .....
OQ372025.1 .....
OQ372026.1 .....
OQ372027.1 .....
OQ372028.1 .....
OQ372029.1 .....

80      90      100     110     120     130     140     150
KT178121.1* CTGAGTACCAAACTAAGGATACTGATATATTGGCAGCAATCCGAGTAACCTCTCAACCTGGAGTTCCACCCTGAAGAAGC
NC_026570.1* CTGAGTACCAAACTAAGGATACTGATATATTGGCAGCAATCCGAGTAACCTCTCAACCTGGAGTTCCACCCTGAAGAAGC
KT178120.1* CTGAGTACCAAACTAAGGATACTGATATATTGGCAGCAATCCGAGTAACCTCTCAACCTGGAGTTCCACCCTGAAGAAGC
NC_048515.1* CTGAGTACCAAACTAAGGATACTGATATATTGGCAGCAATCCGAGTAACCTCTCAACCTGGAGTTCCACCCTGAAGAAGC
NC_048514.1* CTGAGTACCAAACTAAGGATACTGATATATTGGCAGCAATCCGAGTAACCTCTCAACCTGGAGTTCCACCCTGAAGAAGC
NC_070364.1* CTGAGTACCAAACTAAGGATACTGATATATTGGCAGCAATCCGAGTAACCTCTCAACCTGGAGTTCCACCCTGAAGAAGC
NC_039458.1* CTGAGTACCAAACTAAGGATACTGATATATTGGCAGCAATCCGAGTAACCTCTCAACCTGGAGTTCCACCCTGAAGAAGC
NC_039457.1* CTGAGTACCAAACTAAGGATACTGATATATTGGCAGCAATCCGAGTAACCTCTCAACCTGGAGTTCCACCCTGAAGAAGC
OQ507152.1 .....
OQ507153.1 .....
OQ507154.1 .....
OQ507155.1 .....
OQ507156.1 .....
OQ507157.1 .....
OQ507158.1 .....
OQ507159.1 .....
OQ507160.1 .....
OQ507161.1 .....
AY665903.1* .....
AY665868.1* .....
AY665910.1* .....
AY665914.1* .....
AY665879.1* .....
AY665905.1* .....
AY665886.1* .....
MH763728.1* .....
MH763740.1* .....
OQ372021.1 .....
OQ372022.1 .....
OQ372023.1 .....
OQ372024.1 .....
OQ372025.1 .....
OQ372026.1 .....
OQ372027.1 .....
OQ372028.1 .....
OQ372029.1 .....
```

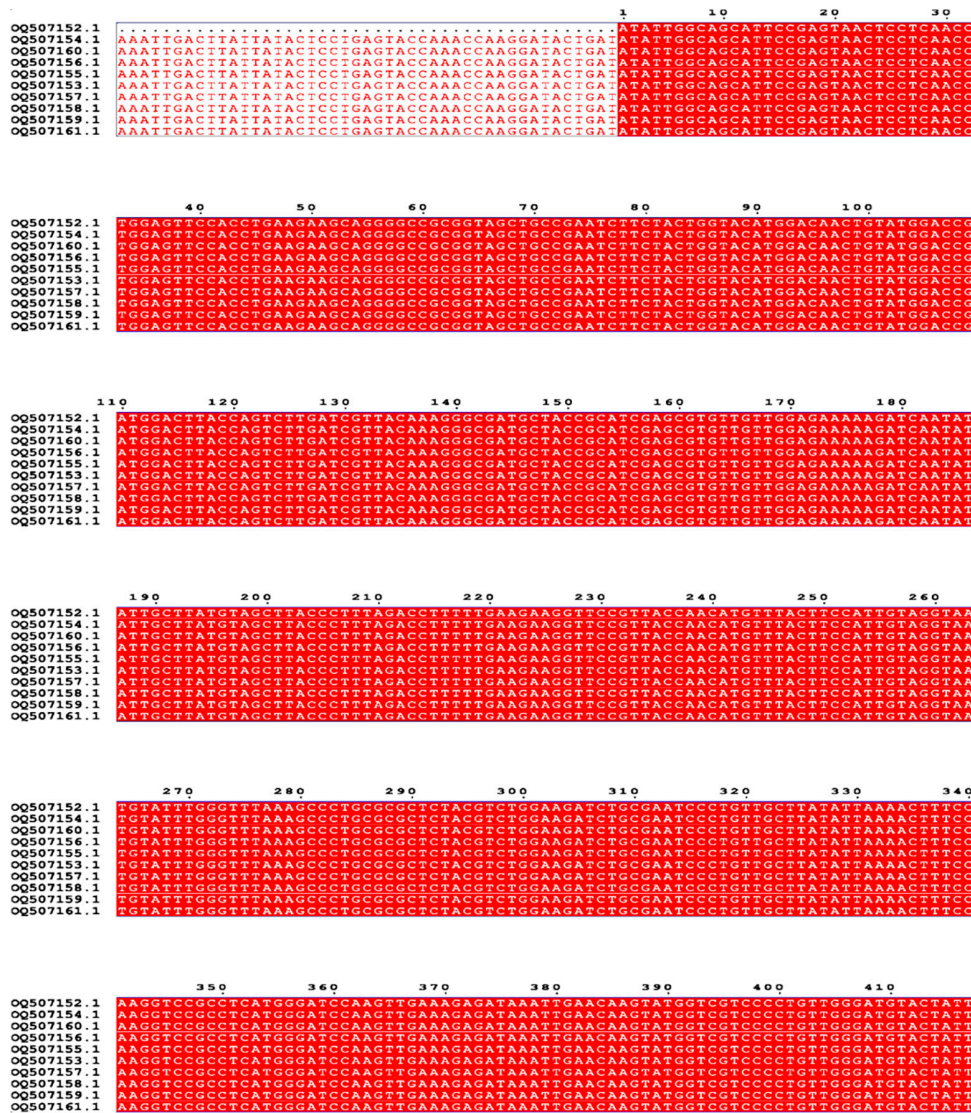

660 670 680 690 700 710 720 730

KT178121.1* TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
NC_026570.1* TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
K018120.1* TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
NC_048145.1* TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
NC_048514.1* TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
NC_070364.1* TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
NC_039458.1* TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
NC_039457.1* TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507152.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507153.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507154.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507155.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507156.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507157.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507158.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507159.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507160.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
OQ507161.1 TTTTGGCGAAGCACTTTTATAAAGCACAGGCTGAAA. CAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGT
AY665903.1* T. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
AY665868.1* G. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
AY665910.1* G. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
AY665914.1* G. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
AY665879.1* G. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
AY665905.1* G. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
AY665886.1* G. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
MH763728.1* G. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
MH763740.1* G. GCGCCTTAGGGCGCTC. CGACCCGCGACCCAGGT. CAGGCGGGGATT.
OQ372021.1 GGTGCGCTTAGGGCGCTT. CGACCCGCGACCCAGGTGAGGTGGAAT.
OQ372022.1 GCTCTGCTTTGTTGGTTT. CGACAAATGATCTCTCC. CAGGTTTACCTT.
OQ372023.1 GCGCTTTAGGGCGCT. CGACCCGCGACCCAGGT. GGGATTTTTTCG.
OQ372024.1 GGTGCGCTTAGGGCGCTT. CGACCCGCGACCCAGGTGAGGTGGAAT.
OQ372025.1 GCTCTGCTTTGTTGGTTT. CGACAAATGATCTCTCC. CAGGTTTACCTT.
OQ372026.1 GGTGCGCTTAGGGCGCTT. CGACAAATGATCTCTCC. CAGGTTTACCTT.
OQ372027.1 GGTGCGCTTAGGGCGCTT. CGACAAATGATCTCTCC. CAGGTTTACCTT.
OQ372028.1 GGTGCGCTTAGGGCGCTT. CGACAAATGATCTCTCC. CAGGTTTACCTT.
OQ372029.1 GGTGCGCTTAGGGCGCTT. CGACAAATGATCTCTCC. CAGGTTTACCTT.

Supplementary Figure S2: Multiple sequence alignment for *Physalis* accessions based on ITS2 gene only (<https://esprict.ibcp.fr/ESPript/temp/1397454355/0-0-1688383432-esp.pdf>)



rbcL gene only (<https://espript.ibcp.fr/ESPript/temp/1129027434/0-0-1688384286-esp.pdf>)



	420	430	440	450	460	470	480	490
Q0507152.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507154.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507160.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507156.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507155.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507153.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507157.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507158.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507159.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							
Q0507161.1	AAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCTGTTTATGAATGCTTC							

	500	510	520	530	540
Q0507152.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507154.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507160.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507156.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507155.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507153.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507157.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507158.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507159.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				
Q0507161.1	CAAAGATGATGAGAACGTGAACCTACAACCATTTATGCGTTGGAGAGATCGTT				

Supplementary Figure S4: Heat map representation of the mineral content of *Physalis* accessions.

Accession Number	[Fe] (ppm)	[Zn] (ppm)	[Ni] (ppm)	[Cu] (ppm)	[Li] (ppm)	[Mn] (ppm)	[Ca] (ppm)	[Na] (ppm)	[K] (ppm)	[Mg] (ppm)
OQ507152.1										
OQ372021.1										
OQ372022.1										
OQ372023.1										
OQ372024.1										
OQ372025.1										
OQ372026.1										
OQ372028.1										
OQ372029.1										

Green color represents specific high mineral concentration for the *Physalis* accession while yellow represents specific moderate mineral concentration and red represents specific low mineral concentration.

Supplementary Figure S5: Heat map for the polyphenol content distribution of *Physalis* accessions

Accession Number	TPC (mg GAE /g DW)	TTC (mg Tannic acid/g DW)	TFC (mg Rutin/g DW)
OQ507152.1	Yellow	Green	Green
OQ372021.1	Orange	Light Green	Yellow
OQ372022.1	Red	Light Green	Yellow
OQ372023.1	Red	Light Green	Orange
OQ372024.1	Red	Dark Green	Light Green
OQ372025.1	Yellow	Orange	Yellow
OQ372026.1	Orange	Orange	Orange
OQ372027.1	Yellow	Orange	Light Green
OQ372028.1	Orange	Red	Green
OQ372029.1	Yellow	Yellow	Green

Green color represents specific high polyphenol concentration for the *Physalis* accession while yellow represents specific moderate polyphenol concentration and red represents specific low polyphenol concentration

Supplementary Figure S6: Heat map for the radical scavenging activity of *Physalis* accessions

Accession Number	DPPH RSA %	HRSA %
OQ507152.1		
OQ372021.1		
OQ372022.1		
OQ372023.1		
OQ372024.1		
OQ372025.1		
OQ372026.1		
OQ372027.1		
OQ372028.1		
OQ372029.1		

Green color represents specific high radical scavenging activity for the *Physalis* accession while yellow represents specific moderate radical scavenging activity and red represents specific low radical scavenging activity.