SUPPLEMENTARY DATA

WITH

Biosynthesis of Cyanogenic Glucosides in *Phaseolus lunatus* and the Evolution of Oxime-based Defenses

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Supplemental Table S1: oligonucleotide primers used in this study.

Degenerate primers for gene cloning.
Primers are designed based on conserved amino acid motifs distinguishing the different CYP gene families in legumes species. DNA sequences from homologous genes from *L. japonicus*, *T. repens* and *P. vulgaris* guided nucleotide selection to account for legume specific codon preference.

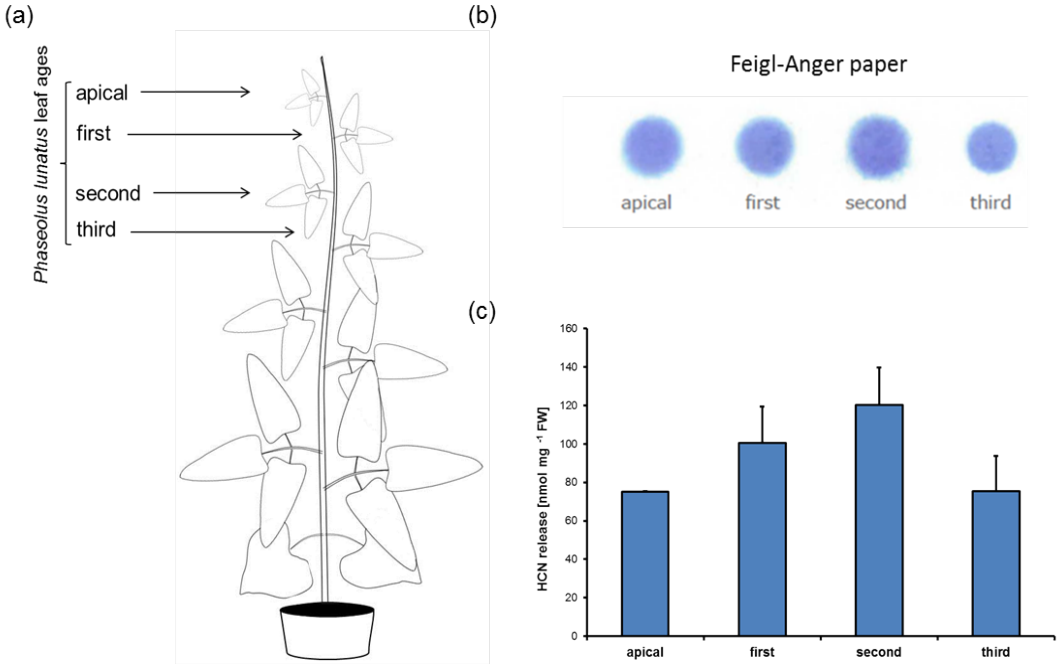
	Forward	**Reverse**
CYP79 (use in 4 combinations possible)	5'-GGYAAYCTYCCTGARATGCTTGCAAA-3' 5'-TGAACACYSAAATWGCNTGTATYCGCCTA-3'	5'-AGCAACTCAGGYTGGTTTAWCATYTCTGC-3' 5'-CKAGCAAAYAASATYAYWGTCATTGTKGT-3'
CYP736	5'-CTYCACATGYTAGGRAAACTMCCACA-3'	5'-TAACATCTTCTCTACACTCRCGAGGTAC-3'
CYP83	5'-CAMAGAAATAYGGNYCTWTATTTTCMCTT-3'	5'-TRTTGTCTTKGCTGGWATTTCRTAARC-3'
UGT85	5'-GTCYCWTGGASMAGAMTTTGTWAAGGG-3'	5'-GAARCCYYTKTCTTTMTCRGGAAAATG-3'

Gene specific primers used in 5' and 3' RACE procedures
Additional (linker) primers are part of the FirstChoice™ RLM-RACE Kit (Ambion).
Primers are used in consecutive PCR reactions (nested PCR).

	5'- RACE	**3'-RACE**
CYP79D71	5'-CACCAAAATGGTGGTTATATATCCTCT-3' 5'-ATGAGATCATTGCACGGGGTTAGTG-3'	5'-CAGTGGACAATCCATCAAATGCTT-3' 5'-GCTTTTGAATGGGCACTAGGTG-3'
CYP79D72	5'-ACAAGGGCTGTGGTGAGATATCCA-3' 5'-ACAAGAGAAGTGGTTATGCTTGTGG-3'	5'-GGTGGATAATCCATCAAATGCAG-3' 5'-GCAGTTGAATGGGGTCTGGCAGA-3'
CYP736A222	5'-TATGGCTGGGACCTGCCCCAGCTTT-3' 5'-GGTTCGATGTGGGAGTTTTCCTAG-3'	5'-AAACATGTGGAGGAAAATGACTTG-3' 5'-AACACTAAGGTTGCACCCGGCTGG-3'
CYP83E46	5'-TCTCTAACCCATTGTAGGACAATT-3' 5'-TTTAGGTGAGGAGATGACAATGGA-3'	5'-GTAAGAAAGAGTTTCTAGAAGAAG-3' 5'-GAGACTGTATCTACCAGCACCATT-3'
CYP83E47	5'-TATCTAACCCATCATAAGACAATC-3' 5'-TTTAGCTGAGGAGACGACAATGGC-3'	5'-GTAAGAAAGAATTTCTAGAAGATC-3' 5'-CAGACTGTATCCAGCAGCACCAAC-3'
UGT85K31	5'-GGTATACCCAAATCCTTCGCAACTTT-3' 5'-CAATTATGCAACTAATTGGAGGCACG-3'	5'-ATAAACACATTCGAAGACTTGGAGGG-3' 5'-TACCATTGGTCCATTTGACTTGCTTG-3'

Gateway cloning PCR primers
The primers additionally contained the attB1 and attB2 Gateway cloning sites: ggggacaagtttgtacaaaaaagcaggct and ggggaccactttgtacaagaaagctgggt. The gene specific sequences (excluding attB1 and attB2 sites) were:

	Forward	**Reverse**
CYP79-A	5'-ATGGAAGATGCTCGTTCTC-3'	5'-TTAAAGGTGATATAAATGT-3'
CYP79-B	5'-ATGGTACAGTATTCTATCT-3'	5'-TCACCCAATATAAAGTTGT-3'
CYP736A222	5'-ATGTTGCCTCAAGAAG-3'	5'- CTAACTAGCTAATCGG -3'
CYP83-A	5'-ATGGTGTCACCACTTCTGA-3'	5'-TTAGATTAGATTTAGATTC-3'
UGT85	5'-ATGGACTCCCTTCAAAGCT-3'	5'-TCAAATAGCCTTGTGGTTT-3'



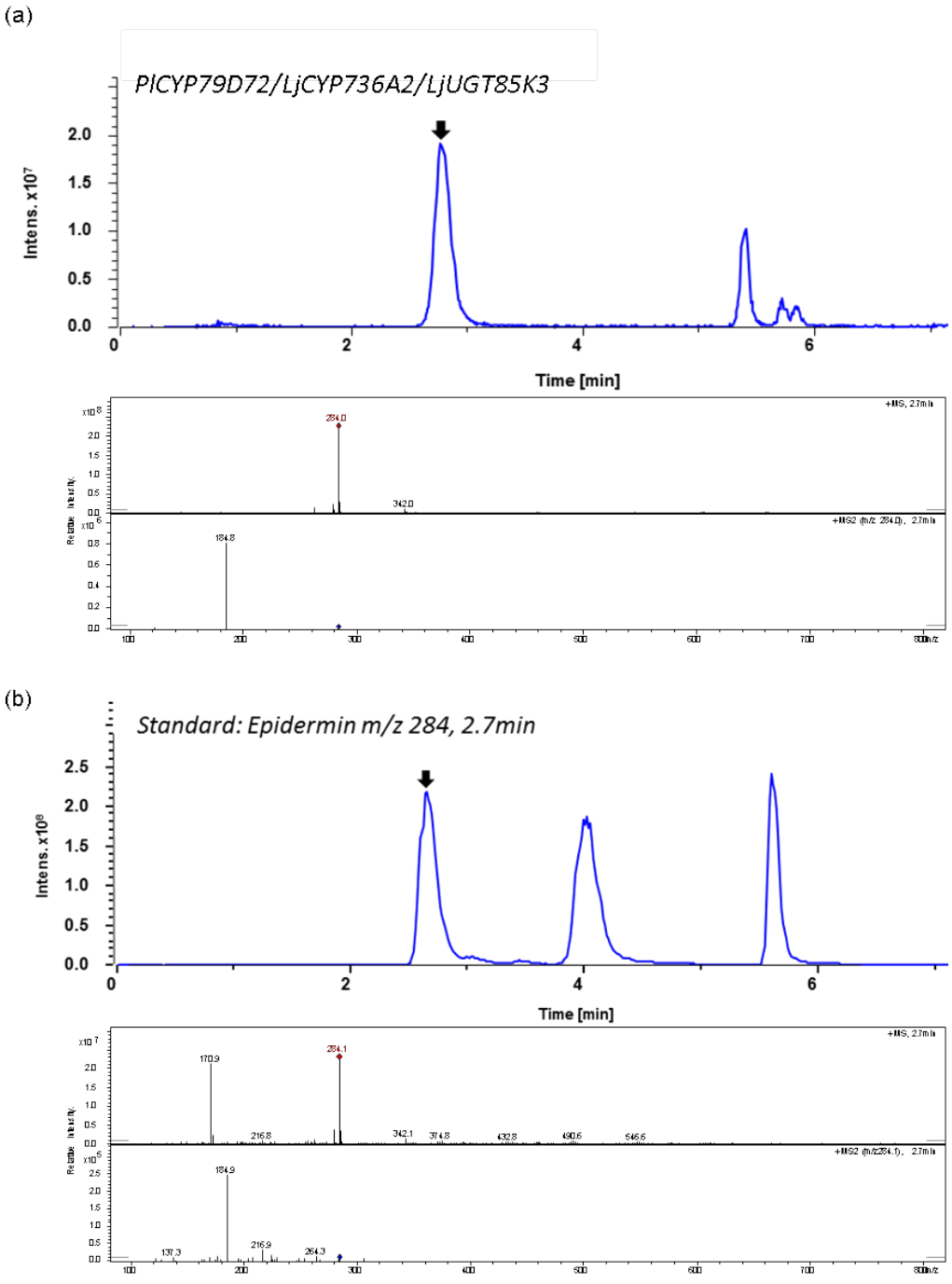
Supplemental Figure S1. Cyanogenic potential in young leaves of *P. lunatus*.
(a) *P. lunatus* plant overview of used leaf ages
(b) Semi-quantitative analysis of HCN release by using HCN-sensitive Feigl-Anger paper
(c) Quantitative analysis of HCN release in the apical, first, second and third leaf of *P. lunatus*. Values are the mean of three technical and three biological replicates ± SE.

Supplemental method:

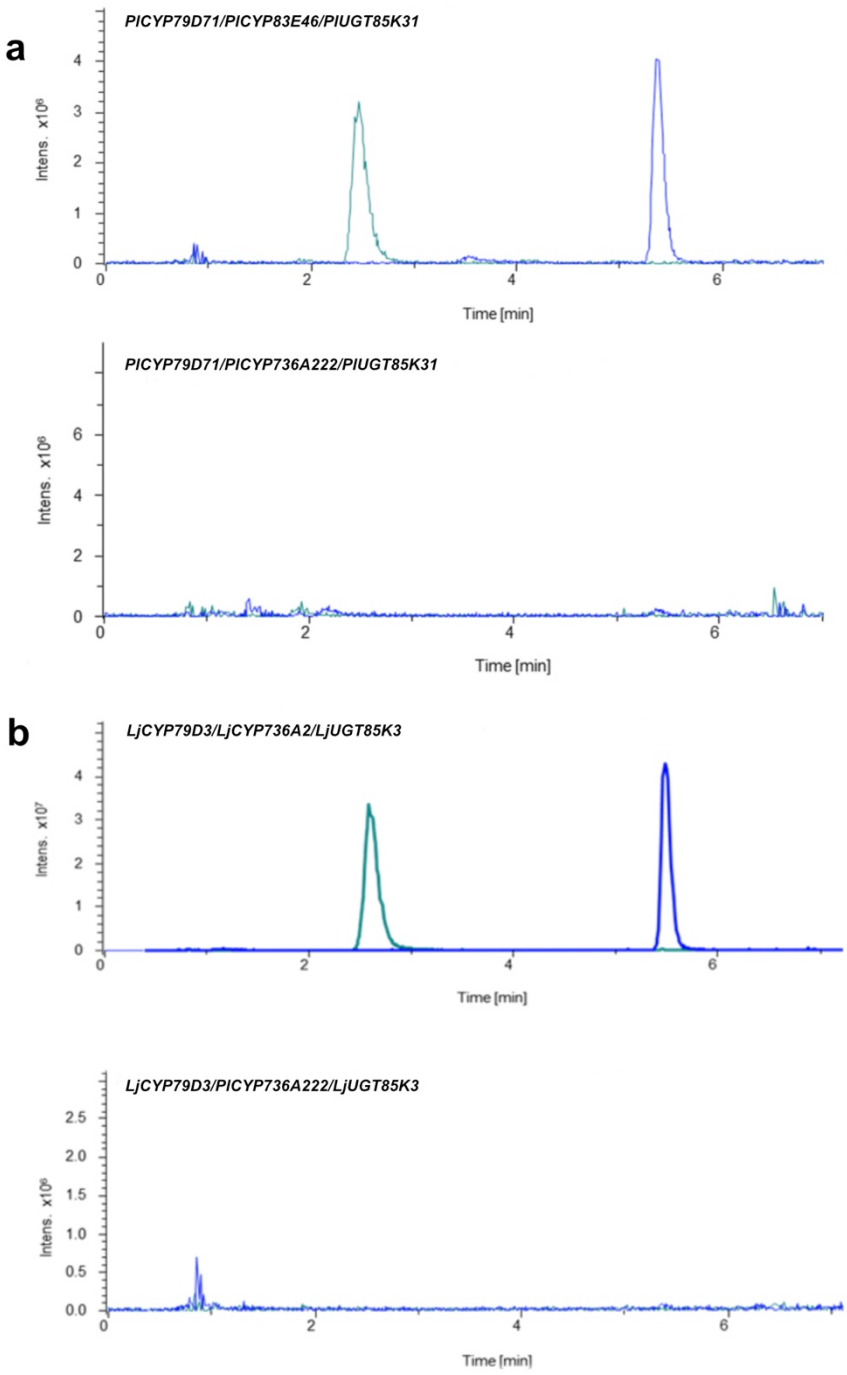
Quantitative HCN detection by using a colorimetric assay

The cyanogenic potential of *P. lunatus* leaves was determined based on the calorimetric method described by Lambert *et al*. (1975) as modified by Halkier & Møller (1989). A small plant sample (5–10 mg) was added to 200 µl MES buffer (20 mM, pH 6.5), freeze/thawed three times on ice, and incubated at 30ºC for 30 min. The incubation was stopped by freezing the samples in liquid nitrogen and addition of NaOH (40 µl, 6 M) to the frozen samples. After thawing up the samples at RT, the hydrogen cyanide potential was determined by addition of glacial HOAc (12.5 µl) immediately followed by 50 µl reagent A (50 mg succinimide and 125 mg *N*-chlorosuccinimide in 50 ml water) and 50 µl reagent B (3 g barbituric acid and 15 ml pyridine in 35 ml water. After thorough mixing and incubation (5 min), the hydrogen cyanide content was determined spectrophotometrically (450-nm to 700-nm scan; UV/VIS spectrometer, Lambda 800; Perkin-Elmer).

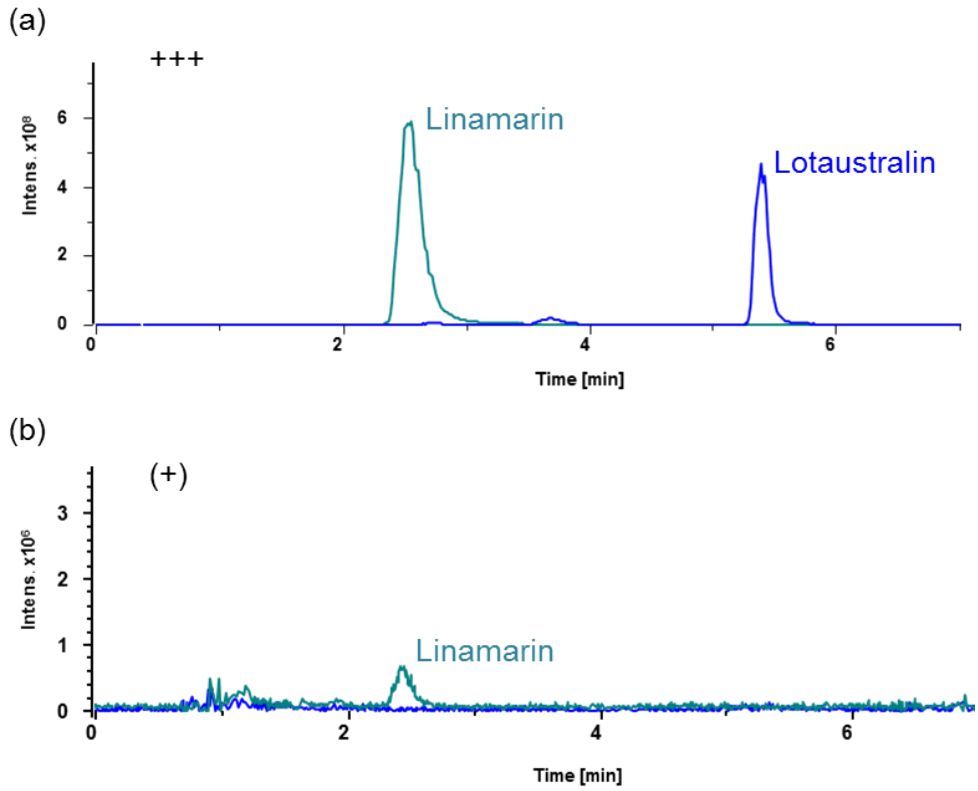
Lambert *et al. Anal. Chem.* **47,** 916–918 (1975).
Halkier, B. a & Møller, B. L. *Plant Physiol.* **90,** 1552–1559 (1989).



Supplemental Figure S2: *Pl*CYP79D72 has leucine as its substrate and in combination with *Lj*CYP736A2 and *Lj*UGT85K3 produces the β-hydroxynitrile glucoside epidermin. (a) Extracted ion chromatogram of tobacco leaves co-infiltrated with the *P. lunatus* gene *CYP79D72* and the *L. japonicus* genes *CYP736A2* and *UGT85K3*. Extracted ion peaks are for sodium adducts: epidermin (m/z 284, RT 2.7 min.) and lotaustralin (m/z 284, RT 5.5 min.). (b) Extracted ion chromatogram of an *Hordeum vulgare* leaf extract. Extracted ion peaks are for sodium adducts: epidermin (m/z 284, RT 2.7 min.), dihydroosmaronin (m/z 284, RT 4.1 min.) and epiheterodendrin (m/z 284, RT 5.6 min.). Mass spectra of the m/z 284 ion and its fragmentation (MS2) are for the epidermin peak (indicated with arrow).



Supplemental Figure S3: CYP736A222 does not substitute for CYP83E46 or CYP736A2 in the production of linamarin and lotaustralin.
Extracted ion chromatogram of tobacco leaves co-infiltrated with (a) the indicated *P. lunatus* gene combinations, or (b) the *L. japonicus* biosynthetic genes in combination with *PlCYP736A222*. Extracted ion peaks are for sodium adducts: linamarin (m/z 270, cyan), lotaustralin (m/z 284, blue).



Supplemental Figure S4: **Examples of differences in cyanogenic glucosides levels in *Phaseolus spp.* accessions.**
Extracted ion chromatogram of two *Phaseolus spp.* accessions with high levels of cyanogenic glucosides in (a) and only trace amounts in (b). Note the 100x difference in the scaling of signal intensity (10^6 versus 10^8). Extracted ion peaks are for sodium adducts: linamarin (m/z 270, cyan) and lotaustralin (m/z 284, blue).

Supplemental Table S2. *Phaseolus spp.* natural and cultivated accessions selected from the CIAT germplasm collection and tested for cyanogenesis and the presence of the cyanogenic glucosides linamarin and lotaustralin in leaf extracts.

[1)] Cyanogenesis was determined by HCN detection with Feigl-Anger paper. An accession was considered polymorphic (or segregating) if separate individual plants showed a different response.
[2)] Relative signal strength in extracted ion chromatograms of cyanogenic glucosides: - = not detectable; (+) = possible trace amount; + = detectable; ++ = clearly present; +++ = abundant.
[3)] Countries of origin: ARG (Argentina), AZE (Azerbaijan), BDI (Burundi), BMU (Bermuda), BOL (Bolivia), BRZ (Brazil), CHL (Chile), CHN (China), CMR (Cameroon), COD (Democratic Republic of the Congo), COL (Colombia), CRI (Costa Rica), CUB (Cuba), DOM (Dominican Republic), ECU (Ecuador), ESP (Spain), ETH (Ethiopia), FRA (France), GHA (Ghana), GTM (Guatemala), HND (Honduras), HTI (Haiti), IND (India), ITA (Italy), JAM (Jamaica), LBR (Liberia), MEX (Mexico), MMR (Myanmar), MWI (Malawi), NGA (Nigeria), NLD (Netherlands), PER (Peru), PHL (Philippines), PRI (Puerto Rico), RWA (Rwanda), SLV (El Salvador), TUR (Turkey), TZA (United Republic of Tanzania), USA (United States of America), ZWB (Zambia).

Species name	CIAT Accession Nr.	Cyanogenesis[1]	Cyanogenic glucosides[2]		Country[3]	Type
			Linamarin	Lotaustralin		
P. acutifolius	G40175	negative	-	-	USA	cultivated
	G40111	negative	-	-	MEX	cultivated
	G40254	negative	-	-	MEX	natural
	G40080	negative	-	-	MEX	natural
	G40163	negative	-	-	MEX	cultivated
	G40299	negative	-	-	GTM	cultivated
	G40283	negative	-	-	USA	cultivated
	G40027	negative	-	-	CRI	cultivated
	G40206	negative	-	-	MEX	natural
P. albescens	G40897	negative	-	-	MEX	natural
P. altimontanus	G40568	negative	-	-	MEX	natural
P. angustissimus	G40685	negative	-	-	USA	natural
P. augusti	G40729	polymorphic	++	++	PER	natural

	G40716	positive	**++**	**+**	ARG	natural
	G40759	positive	**+++**	**++**	BOL	natural
P. carterae	**G40675**	negative	**-**	**-**	MEX	natural
P. chiapasanus	**G40676**	negative	**(+)**	**-**	MEX	natural
P. coccineus	**G35055**	negative	**+**	**-**	MEX	cultivated
	G36136	negative	**-**	**-**	MEX	natural
	G35876	negative	**-**	**-**	GTM	natural
	G35205	negative	**-**	**-**	GTM	cultivated
P. costaricensis	**G40807**	negative	**-**	**-**	CRI	natural
	G40853	negative	**-**	**-**	CRI	natural
	G40894	negative	**+**	**(+)**	CRI	natural
P. dumosus	**G35631**	negative	**-**	**-**	GTM	cultivated
	G35314	negative	**-**	**-**	COL	cultivated
	G35550	negative	**-**	**-**	MEX	cultivated
	G35855	negative	**-**	**-**	GTM	natural
	G35584	negative	**-**	**-**	GTM	cultivated
P. esperanzae	**G40817**	negative	**(+)**	**-**	MEX	natural
P. filiformis	**G40512**	negative	**-**	**-**	USA	natural
P. glabellus	**G40797**	negative	**(+)**	**-**	MEX	natural
P. grayanus	**G40517**	negative	**-**	**-**	MEX	natural
P. lepstostachyus	**G40620**	negative	**-**	**-**	MEX	natural
P. lignosus	**G40876**	positive	**+++**	**++**	BMU	natural
P. lunatus	**G26191**	polymorphic	**++**	**+**	NGA	cultivated
	G25253	polymorphic	**++**	**++**	CMR	cultivated
	G26193	negative	**-**	**-**	COD	cultivated
	G25694	polymorphic	**+++**	**+++**	ETH	cultivated
	G26195	polymorphic	**++**	**++**	RWA	cultivated
	G25237	polymorphic	**++**	**+**	IND	cultivated

	G27616	positive	**+++**	**++**	IND	cultivated
	G25247D	positive	**+++**	**++**	GHA	cultivated
	G27209	positive	**+++**	**++**	LBR	cultivated
	G25969	polymorphic	**++**	**+**	CHN	cultivated
	G25820	positive	**+++**	**++**	PHL	cultivated
	G25625	polymorphic	**+++**	**++**	USA	cultivated
	G25799	positive	**+++**	**++**	MEX	cultivated
	G26534	polymorphic	**+++**	**++**	MEX	cultivated
	G27399	polymorphic	**+++**	**++**	MEX	cultivated
	G25975	positive	**+++**	**++**	GTM	cultivated
	G25295	polymorphic	**+++**	**++**	CUB	cultivated
	G27553	positive	**+++**	**++**	DOM	cultivated
	G25616	positive	**+++**	**++**	HTI	cultivated
	G25869	polymorphic	**+++**	**++**	COL	cultivated
	G27309	positive	**+++**	**++**	ECU	cultivated
	G27416	positive	**+++**	**+++**	PER	cultivated
	G25990	polymorphic	**+++**	**++**	BOL	cultivated
	G25785	positive	**++**	**+**	MEX	natural
	G26517	positive	**+++**	**++**	MEX	natural
	G26360	positive	**+++**	**++**	MEX	natural
	G26681	positive	**++**	**++**	GTM	natural
	G26629	polymorphic	**+**	**-**	HND	natural
	G25294D	positive	**+++**	**+++**	CUB	natural
	G27339	positive	**++**	**+**	PRI	natural
	G27609	positive	**++**	**+**	SLV	natural
	G26606	polymorphic	**+++**	**++**	ECU	natural
	G26348	positive	**++**	**+**	PER	natural
	G26547	positive	**+++**	**+**	PER	natural

	G25392C	positive	**++**	**+**	CRI	cultivated
	G25294B	positive	**++**	**+**	CUB	cultivated
	G26164	polymorphic	**++**	**+**	MEX	cultivated
	G26221	positive	**+++**	**++**	USA	cultivated
	G25872J	positive	**++**	**+**	COL	cultivated
	G25372A	positive	**++**	**+**	SLV	cultivated
	G25007	polymorphic	**+++**	**++**	BRZ	cultivated
	G27580A	positive	**+++**	**++**	ZWB	cultivated
	G25697	positive	**++**	**+**	ECU	cultivated
	G25219	positive	**+**	**(+)**	MEX	natural
	G25541C	positive	**++**	**+**	ARG	cultivated
	G26615	positive	**+++**	**++**	COL	natural
	G25575B	positive	**+++**	**++**	PER	cultivated
	G25713A	positive	**+++**	**++**	MEX	natural
	G25695	positive	**+++**	**++**	PHL	natural
	G27519	positive	**+**	**-**	HTI	cultivated
	G26468	polymorphic	**++**	**+**	ECU	natural
	G25299	positive	**++**	**+**	IND	cultivated
	G26533	negative	**++**	**+**	MEX	natural
	G25255A	polymorphic	**+++**	**++**	ETH	cultivated
	G26459	polymorphic	**++**	**+**	ECU	natural
	G26653	negative	**++**	**+**	GTM	natural
	G25246A	positive	**++**	**+**	GHA	cultivated
	G25915	polymorphic	**+++**	**+**	PER	natural
	G27414	positive	**++**	**+**	MEX	natural
	G26767	positive	**++**	**+**	LBR	cultivated
	G27573	polymorphic	**++**	**+**	GTM	natural
	G25578	positive	**++**	**+**	BOL	cultivated

	G27465	positive	**+++**	**+**	MMR	cultivated
	G25913	polymorphic	**++**	**+**	PER	natural
	G25239	polymorphic	**++**	**+**	GTM	cultivated
	G27574	polymorphic	**++**	**+**	AZE	cultivated
	G25388A	positive	**++**	**+**	CRI	natural
	G25540	positive	**++**	**+**	ARG	cultivated
	G26805	polymorphic	**++**	**+**	NGA	cultivated
	G26749	negative	**+**	**+**	ARG	natural
	G25315	negative	**+++**	**++**	JAM	cultivated
P. macrolepis	**G40863**	negative	**(+)**	**-**	GTM	natural
P. maculatus	**G40809**	positive	**++**	**+**	MEX	natural
P. macvaughii	**G40760**	negative	**-**	**(+)**	MEX	natural
P. magnilobatus	**G40514**	negative	**(+)**	**-**	MEX	natural
P. marechalii	**G40812**	positive	**+**	**++**	MEX	natural
P. microcarpus	**G40538**	negative	**-**	**-**	MEX	natural
P. nodosus	**G40899**	positive	**+**	**++**	MEX	natural
P. oaxacanus	**G40861**	negative	**-**	**-**	MEX	natural
P. oligospermus	**G40595**	negative	**-**	**-**	CRI	natural
P. pachyrrhizoides	**G40770**	positive	**+++**	**++**	PER	natural
P. parvifolius	**G40185**	negative	**+**	**-**	GTM	natural
P. pedicellatus	**G40589**	negative	**-**	**-**	MEX	natural
P. polystachios	**G40783**	positive	**+**	**+**	USA	natural
P. pluriflorus	**G40532**	negative	**-**	**-**	MEX	natural
P. rotundatus	**G40728**	positive	**++**	**++**	MEX	natural
P. salicifolius	**G40860**	positive	**+**	**+**	MEX	natural
P. talamancenis	**G40814**	negative	**-**	**-**	CRI	natural
	G11032A	negative	**-**	**-**	MEX	natural
P. vulgaris	**G12866A**	negative	**(+)**	**-**	MEX	natural

	G12882B	negative	-	-	MEX	natural
	G24576	negative	**(+)**	-	MEX	natural
	G24323	negative	-	-	MEX	natural
	G50505A	negative	-	-	GTM	natural
	G23436	negative	-	-	GTM	natural
	G51062	negative	-	-	CRI	natural
	G24798	negative	-	-	COL	natural
	G23582	negative	-	-	ECU	natural
	G23583	negative	-	-	PER	natural
	G19889	negative	-	-	ARG	natural
	G16392	negative	-	-	USA	cultivated
	G11108	negative	-	-	MEX	cultivated
	G9712	negative	-	-	MEX	cultivated
	G6992	negative	-	-	MEX	cultivated
	G79	negative	-	-	SLV	cultivated
	G23980	negative	-	-	COL	cultivated
	G18324	negative	-	-	PER	cultivated
	G12031	negative	-	-	PER	cultivated
	G23769D	negative	-	-	PER	cultivated
	G50468C	negative	**(+)**	-	BOL	cultivated
	G18355	negative	-	-	CHL	cultivated
	G13942	negative	-	-	ARG	cultivated
	G1477	negative	-	-	ESP	cultivated
	G8013	negative	-	-	FRA	cultivated
	G18048	negative	-	-	NLD	cultivated
	G3815	negative	**(+)**	-	ITA	cultivated
	G15568	negative	-	-	TUR	cultivated
	G766	negative	-	-	ETH	cultivated

	G19151A	negative	-	-	TZA	cultivated
	G22534	negative	-	-	BDI	cultivated
	G15366	negative	-	-	MWI	cultivated
	G467	negative	-	-	IND	cultivated
	G24279	negative	-	-	CHN	cultivated
	G24317	negative	-	-	CHN	cultivated
	G23470	negative	-	-	MEX	natural
	G10655	negative	-	-	GTM	cultivated
	G21117	negative	-	-	COL	natural
	G23617A	negative	-	-	BOL	cultivated
	G6386	negative	-	-	MEX	natural
	G2756	negative	-	-	HND	cultivated
	G23725	negative	-	-	ECU	natural
	G21044A	negative	-	-	ARG	cultivated
	G24382	negative	-	-	MEX	natural
	G51648	negative	-	-	CRI	cultivated
	G23421	negative	-	-	PER	natural
	G4830	negative	-	-	BRZ	cultivated
	G12855A	negative	-	-	GTM	natural
	G51281	negative	-	-	COL	cultivated
	G23458	negative	-	-	PER	natural
	G5818	negative	-	-	CHL	cultivated
	G23478	negative	-	-	GTM	natural
	G23445B	negative	-	-	BOL	natural
	G23416	negative	+	-	CRI	natural
	G19898	negative	-	-	ARG	natural
	G19918	negative	-	-	MEX	cultivated
	G12361	negative	-	-	ECU	cultivated

	G2433	negative	-	-	MEX	cultivated
	G12120	negative	-	-	PER	cultivated
P. zimapanensis	**G40556**	negative	-	-	MEX	natural