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Rubisco catalytic properties and temperature response in crops.

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1 **Table S1.** The Rubisco kinetic parameters measured at 15 °C and 35 °C for the selected crop species. The Michaelis-Menten affinity constants for CO₂ under
 2 non-oxygenic (K_c) and 21% O₂ (K_c^{air}), the maximum carboxylation rate (k_{cat}^c), the specificity factor ($S_{c/o}$) and the carboxylation (k_{cat}^c/K_c) and the oxygenation
 3 catalytic efficiencies (k_{cat}^o/K_o). The ratio k_{cat}^o/K_o was calculated as $(k_{\text{cat}}^c/K_c)/S_{c/o}$. For each species, data are mean \pm standard error (n = 3-9). Group averages
 4 were obtained from individual measurements on each species. Different letters denote statistical differences ($p < 0.05$) by Duncan analysis between C₃ and C₄
 5 groups.

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	K_c		K_c^{air}		k_{cat}^c		$S_{c/o}$		k_{cat}^c/K_c		k_{cat}^o/K_o	
	(μM)		(μM)		(s ⁻¹)		(mol mol ⁻¹)		(s ⁻¹ μM^{-1})		(s ⁻¹ nM ⁻¹)	
	15 °C	35 °C	15 °C	35 °C	15 °C	35 °C	15 °C	35 °C	15 °C	35 °C	15 °C	35 °C
C ₃ species												
<i>Avena sativa</i>	6.0 \pm 0.6	19.3 \pm 1.1	13.2 \pm 1.6	29.5 \pm 2.7	1.3 \pm 0.0	3.9 \pm 0.1	124.0 \pm 3.6	77.6 \pm 1.1	0.21 \pm 0.02	0.20 \pm 0.01	1.70 \pm 0.17	2.61 \pm 0.08
<i>Beta vulgaris</i>	5.2 \pm 0.4	16.1 \pm 0.4	9.6 \pm 0.9	27.0 \pm 0.9	1.3 \pm 0.1	4.5 \pm 0.1	117.6 \pm 6.4	79.6 \pm 0.1	0.27 \pm 0.03	0.28 \pm 0.01	2.33 \pm 0.18	3.51 \pm 0.16
<i>Brassica oleracea</i>	5.2 \pm 0.2	17.1 \pm 1.2	11.3 \pm 1.9	27.4 \pm 0.7	1.1 \pm 0.2	3.9 \pm 0.1	116.1 \pm 0.4	76.2 \pm 1.6	0.21 \pm 0.03	0.23 \pm 0.01	1.84 \pm 0.24	2.99 \pm 0.16
<i>Capsicum annuum</i>	6.4 \pm 0.4	15.0 \pm 1.4	11.1 \pm 0.7	28.9 \pm 4.0	0.8 \pm 0.0	2.7 \pm 0.3	128.6 \pm 2.5	79.1 \pm 1.4	0.12 \pm 0.00	0.19 \pm 0.03	0.95 \pm 0.03	2.41 \pm 0.41
<i>Coffea arabica</i>	7.3 \pm 0.6	23.0 \pm 0.8	13.1 \pm 0.8	36.4 \pm 4.3	1.5 \pm 0.2	3.9 \pm 0.8	121.4 \pm 2.5	76.4 \pm 3.8	0.21 \pm 0.04	0.17 \pm 0.04	1.72 \pm 0.29	2.28 \pm 0.48
<i>Cucurbita maxima</i>	6.4 \pm 0.2	15.7 \pm 1.2	12.1 \pm 1.2	31.1 \pm 0.9	0.5 \pm 0.0	3.3 \pm 0.2	132.2 \pm 6.0	84.7 \pm 2.0	0.08 \pm 0.00	0.21 \pm 0.03	0.64 \pm 0.04	2.52 \pm 0.30
<i>Glycine max</i>	3.6 \pm 0.4	16.2 \pm 0.4	7.4 \pm 0.2	23.8 \pm 2.2	0.8 \pm 0.1	3.3 \pm 0.3	126.4 \pm 4.1	75.3 \pm 7.2	0.25 \pm 0.06	0.21 \pm 0.04	1.91 \pm 0.46	2.92 \pm 0.84
<i>Hordeum vulgare</i>	5.3 \pm 0.8	15.8 \pm 0.8	9.6 \pm 0.9	26.2 \pm 0.6	1.8 \pm 0.4	3.7 \pm 0.2	120.6 \pm 6.3	77.5 \pm 1.7	0.34 \pm 0.02	0.24 \pm 0.00	2.81 \pm 0.32	3.04 \pm 0.07

<i>Ipomoea batatas</i>	7.6 ± 0.8	16.1 ± 1.0	12.8 ± 0.5	24.7 ± 2.5	1.2 ± 0.2	3.4 ± 0.1	126.0 ± 3.6	76.3 ± 1.7	0.15 ± 0.03	0.21 ± 0.01	1.21 ± 0.26	2.70 ± 0.12
<i>Lactuca sativa</i>	7.1 ± 0.7	16.0 ± 1.0	9.5 ± 0.5	28.2 ± 3.1	1.2 ± 0.0	3.1 ± 0.2	118.2 ± 1.8	78.2 ± 1.8	0.17 ± 0.01	0.17 ± 0.01	1.41 ± 0.10	2.20 ± 0.07
<i>Manihot esculenta</i>	2.0 ± 0.1	11.0 ± 1.0	3.9 ± 0.4	20.7 ± 0.3	0.9 ± 0.0	2.7 ± 0.2	128.6 ± 2.7	85.0 ± 0.8	0.47 ± 0.02	0.24 ± 0.01	3.55 ± 0.25	2.80 ± 0.18
<i>Medicago sativa</i>	5.1 ± 0.4	13.6 ± 1.0	10.2 ± 0.2	22.0 ± 0.6	0.6 ± 0.2	3.2 ± 0.1	120.1 ± 0.6	80.4 ± 0.6	0.13 ± 0.03	0.23 ± 0.01	1.05 ± 0.23	2.91 ± 0.20
<i>Oryza sativa</i>	2.1 ± 0.1	17.3 ± 1.4	6.4 ± 0.3	25.5 ± 1.4	0.6 ± 0.0	3.0 ± 0.0	123.7 ± 2.5	74.2 ± 1.4	0.27 ± 0.04	0.17 ± 0.01	2.31 ± 0.39	2.33 ± 0.14
<i>Phaseolus vulgaris</i>	4.1 ± 0.1	14.1 ± 0.6	7.2 ± 0.4	23.7 ± 1.8	0.9 ± 0.0	3.3 ± 0.3	123.3 ± 3.1	75.5 ± 1.8	0.23 ± 0.02	0.23 ± 0.03	1.84 ± 0.09	3.01 ± 0.20
<i>Solanum lycopersicum</i>	6.4 ± 1.8	17.8 ± 0.2	9.0 ± 1.8	34.7 ± 0.7	1.3 ± 0.1	3.4 ± 0.2	119.0 ± 1.0	77.1 ± 0.7	0.23 ± 0.07	0.19 ± 0.01	1.97 ± 0.61	2.46 ± 0.14
<i>Solanum tuberosum</i>	7.6 ± 0.4	12.3 ± 0.3	12.9 ± 0.3	25.2 ± 1.0	1.0 ± 0.1	3.0 ± 0.4	125.0 ± 1.4	77.6 ± 4.9	0.07 ± 0.01	0.25 ± 0.03	0.58 ± 0.08	3.35 ± 0.45
<i>Spinacia oleracea</i>	8.9 ± 0.4	29.1 ± 0.4	17.6 ± 0.7	36.8 ± 1.8	0.9 ± 0.1	4.1 ± 0.2	130.1 ± 1.0	76.7 ± 1.9	0.10 ± 0.00	0.14 ± 0.00	0.77 ± 0.01	1.81 ± 0.10
<i>Triticum aestivum</i>	4.5 ± 0.2	18.1 ± 0.8	9.1 ± 0.7	30.8 ± 3.6	1.1 ± 0.1	3.5 ± 0.2	128.2 ± 3.0	78.1 ± 1.9	0.23 ± 0.02	0.20 ± 0.02	2.01 ± 0.13	2.77 ± 0.15
<i>C₃ average</i>	5.5 ± 0.3 ^a	17.0 ± 0.6 ^a	10.4 ± 0.4 ^a	28.0 ± 0.81 ^a	1.0 ± 0.1 ^a	3.5 ± 0.1 ^a	124.7 ± 0.9 ^a	78.2 ± 0.6 ^a	0.21 ± 0.01 ^a	0.21 ± 0.01 ^a	1.71 ± 0.11 ^a	2.70 ± 0.08 ^a
C ₄ species												
<i>Saccharum × officinarum</i>	17.5 ± 0.8	44.9 ± 0.9	18.6 ± 2.5	58.2 ± 2.8	2.8 ± 0.2	5.7 ± 0.2	115.4 ± 2.7	65.65 ± 2.1	0.16 ± 0.02	0.14 ± 0.01	1.41 ± 0.20	2.08 ± 0.19
<i>Zea mays</i>	19.6 ± 1.4	39.0 ± 1.4	26.3 ± 3.9	52.1 ± 2.0	2.4 ± 0.5	5.7 ± 1.1	113.3 ± 1.7	68.5 ± 1.1	0.09 ± 0.02	0.10 ± 0.00	0.79 ± 0.2	1.50 ± 0.03
<i>C₄ average</i>	18.3 ± 1.6 ^b	42.3 ± 1.3 ^b	22.1 ± 2.5 ^b	55.9 ± 2.1 ^b	2.6 ± 0.3 ^b	5.7 ± 0.7 ^b	114.5 ± 1.6 ^b	66.9 ± 1.3 ^b	0.13 ± 0.02 ^b	0.12 ± 0.01 ^b	1.20 ± 0.2 ^b	1.83 ± 0.16 ^b

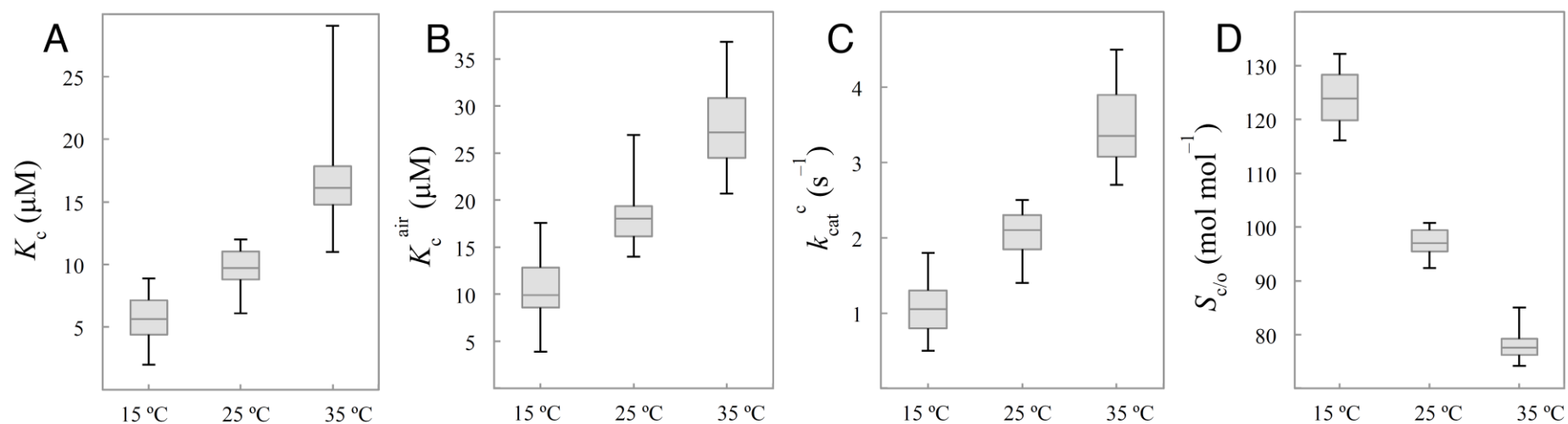
9 **Table S2.** List of crop species with GenBank accession numbers for *rbcL*, *matK* and *ndhF*
10 chloroplast genes examined in the present study. Note that for many species these sequences
11 were extracted from full chloroplast genome sequences, which explains identical accession
12 for the three genes.

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Species	Accession No.		
	<i>rbcL</i>	<i>matK</i>	<i>ndhF</i>
<i>Avena sativa</i>	L15300	AF164395	DQ786814
<i>Beta vulgaris</i>	DQ067450	DQ116790	KJ081864
<i>Brassica oleracea</i>	M88342	155676616	DQ288742
<i>Capsicum annuum</i>	404474503	404474503	404474503
<i>Coffea arabica</i>	EF044213	EF044213	EF044213
<i>Cucurbita maxima</i>	HQ438627	HQ438602	JF412791
<i>Glycine max</i>	91214122	91214122	91214122
<i>Hordeum vulgare</i>	KC912687	KC912687	KC912687
<i>Ipomoea batatas</i>	KF242475	KF242475	KF242475
<i>Lactuca sativa</i>	78675147	78675147	78675147
<i>Manihot esculenta</i>	EU117376	EU117376	EU117376
<i>Medicago sativa</i>	X04975	AY386881	JX512024
<i>Oryza sativa</i>	AY522331	AY522331	AY522331
<i>Phaseolus vulgaris</i>	EU196765	EU196765	EU196765
<i>Saccharum × officinarum</i>	49659489	49659489	49659489
<i>Solanum lycopersicum</i>	544163592	544163592	544163592
<i>Solanum tuberosum</i>	DQ386163	DQ386163	DQ386163
<i>Spinacia oleracea</i>	7636084	7636084	7636084
<i>Triticum aestivum</i>	KJ592713	KJ592713	KJ592713
<i>Zea mays</i>	11994090	11994090	11994090

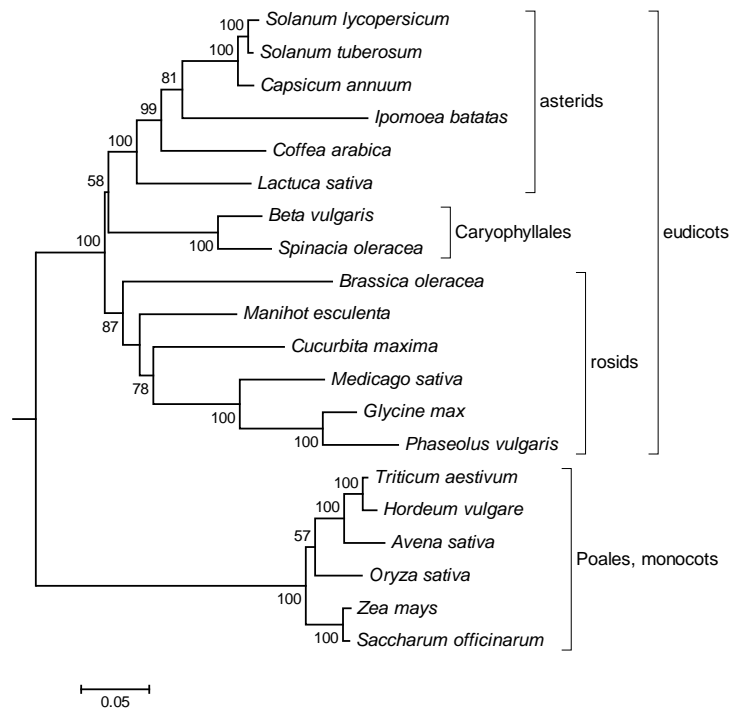
35 **Figure S1.** Box plots depiction of Rubisco kinetic parameters at 15 °C, 25 °C and 35 °C when considering the 18 C₃ species alone: (A) the Michaelis-Menten
36 affinity constant for CO₂ under non-oxygenic condition (K_c) and (B) under 21% O₂ (K_c^{air}), (C) the maximum carboxylation rate (k_{cat}^c), and (D) the specificity
37 factor ($S_{c/o}$). The values used for the representation are those shown in Tables 1 and S2. The box and whisker represent the 25 to 75 percentile and minimum-
38 maximum distributions of the data, respectively.

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43 **Figure S2.** Maximum likelihood phylogeny constructed using concatenated alignment of *rbcL*, *matK* and *ndhF* chloroplastic genes from GenBank for 20
 44 species of crops. It was conducted with RAxML version 7.2.6 (Stamatakis 2006). Numbers near branches are bootstrap support values (%). The species are
 45 grouped into monocots and eudicots.



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47 **Stamatakis A** (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

48 **Figure S3.** Rubisco large subunit (L-subunit) amino acid alignment for the 20 crops species. Hyphens at the site 469 indicate an insertion/deletion. Residues
 49 identical to those of the first sequence are shown as dots. All *rbcL* sequences were taken from GenBank and accession numbers are given in Table S1.

Species	10	14	19	21	23	28	30	32	43	46	50	68	76	86	87	89	91	93	94	95	97	99	114	117	131	140	142	143	145	153	189	203
<i>A. sativa</i>	S	Q	D	K	T	E	E	K	T	P	P	T	S	H	I	P	A	E	D	N	W	C	T	F	R	I	P	A	T	H	C	D
<i>B. vulgaris</i>	.	K	L	S	E	.	Y	V	.	V	.	.	N	
<i>B. oleracea</i>	.	K	E	.	N	P	.	E	T	F	A	.	.	A	
<i>C. annuum</i>	.	K	E	.	.	.	Q	R	.	R	V	.	K	D	Y	A	.	.	.	V	T	.	I	.	.	
<i>C. arabica</i>	.	K	E	A	P	.	E	.	Y	A	.	.	.	V	.	.	I	.	.	
<i>C. maxima</i>	.	K	G	.	.	P	.	E	.	Y	A	T	.	I	.	.	
<i>G. max</i>	.	K	.	.	.	D	G	L	.	.	.	E	.	Y	A	T	.	I	.	.	
<i>H. vulgare</i>	G	S	S	T	S	.	C	.	
<i>I. batatas</i>	.	K	Q	R	.	R	I	.	K	D	Y	A	T	.	I	.	.	
<i>L. sativa</i>	.	K	G	.	.	P	.	E	.	Y	A	T	.	V	.	.	
<i>M. esculenta</i>	.	K	.	.	.	D	Q	G	L	.	P	.	E	.	Y	A	.	.	.	V	.	.	S	.	.	
<i>M. sativa</i>	T	K	.	R	.	D	.	S	.	A	E	T	F	A	N	.	.	.	A	.	V	Q	.	
<i>O. sativa</i>	.	K	V	.	.	.	Y	A	T	S	.	C	.	
<i>P. vulgaris</i>	.	K	.	.	.	D	E	.	F	A	T	.	I	.	.	
<i>S. lycopersicum</i>	.	K	E	.	.	.	Q	R	.	R	V	.	K	D	Y	A	V	.	.	
<i>S. tuberosum</i>	.	K	E	.	.	.	Q	R	.	R	V	.	K	D	Y	A	.	L	.	.	V	.	V	.	.	
<i>S. oleracea</i>	.	K	L	S	.	.	.	N	E	.	Y	V	.	V	.	.		
<i>T. aestivum</i>	G	K	S	S	T	S	.	C	.	

S. × officinarum . K D L P D P D Y V . C .
Z. mays . K D L P D P D Y S . C .

Species	219	225	226	228	230	249	251	255	256	258	262	270	272	279	281	309	317	320	326	328	338	340	341	353	354	356	359	363
<i>A. sativa</i>	V	I	Y	A	A	E	I	V	F	R	V	I	G	S	A	M	A	M	I	S	E	E	M	F	I	K	A	F
<i>B. vulgaris</i>	L	L	L	.	T	.	.	.	L	.	.	.	D	I	Y	T	.	S	Y	
<i>B. oleracea</i>	L	.	.	S	.	.	M	I	.	.	L	L	V	A	D	.	S	Y	V	.	S	.	
<i>C. annuum</i>	L	L	F	.	T	.	M	.	.	.	A	L	A	.	D	I	.	V	Q	S	Y
<i>C. arabica</i>	C	L	F	L	G	.	.	A	.	D	I	.	.	.	S	Y	
<i>C. maxima</i>	L	.	.	S	.	.	M	I	.	.	L	L	.	A	.	D	I	.	V	.	S	Y	
<i>G. max</i>	L	.	F	S	.	.	M	.	.	.	L	L	V	A	.	.	I	.	V	.	S	Y	
<i>H. vulgare</i>	.	.	.	S	L	.	T
<i>I. batatas</i>	L	L	M	I	.	.	L	L	.	A	.	.	I	.	V	Q	S	Y	
<i>L. sativa</i>	L	.	F	S	.	.	M	I	.	.	L	.	.	.	I	I	.	.	.	S	Y	
<i>M. esculenta</i>	L	C	.	L	L	.	A	.	D	I	.	.	.	S	Y	
<i>M. sativa</i>	L	M	.	.	.	L	V	T	A	.	D	I	.	.	.	S	.	
<i>O. sativa</i>	.	.	.	S	L	A	
<i>P. vulgaris</i>	L	L	L	V	.	.	.	I	.	.	.	S	Y	
<i>S. lycopersicum</i>	L	L	F	.	T	L	.	T	.	I	D	I	.	V	Q	S	Y
<i>S. tuberosum</i>	L	L	F	.	.	.	M	.	.	T	L	.	T	D	I	.	.	Q	S	Y
<i>S. oleracea</i>	L	L	.	.	.	D	M	.	.	.	L	.	T	S	.	.	L	D	I	Y	T	.	S	Y
<i>T. aestivum</i>	.	.	.	S	L	.	T
<i>S. × officinarum</i>	K	L	.	T	S	I	.	.	.	S	.
<i>Z. mays</i>	L	.	T	S	I	.	.	.	S	.

Species	367	371	375	418	429	436	439	443	446	447	449	453	456	461	464	466	468	469	471	472	473	475	476	477	478	479	480	481
<i>A. sativa</i>	D	M	I	A	Q	D	R	E	R	E	C	P	A	V	A	K	E	-	E	P	V	T	I	D	E			
<i>B. vulgaris</i>	S	T	L	V	.	N	T	A	.	.	S	.	.	.	E	.	E	-	P	A	M	.	V					
<i>B. oleracea</i>	.	L	L	V	.	.	V	E	T	N	-	P	T	I	K	L	D	G	Q	D	
<i>C. annuum</i>	.	L	L	V	K	S	.	.	.	E	V	N	-	A	A	.	V	L	D	K			
<i>C. arabica</i>	.	L	.	V	K	.	A	.	.	.	S	.	.	.	E	R	N	-	.	A	M	K	L	D	K	E	K	D
<i>C. maxima</i>	.	L	L	V	S	E	-	.	A	.	.	L					
<i>G. max</i>	.	L	L	V	S	.	.	.	E	.	E	-	.	A	M	.	L					
<i>H. vulgare</i>	A	E	-	D	K	K	V	
<i>I. batatas</i>	.	L	L	V	S	.	E	R	E	-	K	.	.	.	L	D	P	G	T	A
<i>L. sativa</i>	.	L	L	V	.	.	T	.	.	.	T	.	.	.	E	.	E	-	Q	A	M	.	L	D	Q			
<i>M. esculenta</i>	.	L	L	V	.	.	.	D	.	.	S	.	.	.	E	.	E	-	A	A	.	.	L	D	K			
<i>M. sativa</i>	.	L	L	V	T	.	.	.	E	.	E	-	P	A	M	N						
<i>O. sativa</i>	S	.	.	.	I	.	.	E	-	.	.	.	K	L	D	S			
<i>P. vulgaris</i>	.	L	L	V	S	.	.	.	E	.	E	-	.	A	M	.	L	D				
<i>S. lycopersicum</i>	.	L	L	V	K	E	V	N	-	A	A	.	V	L	D	K			
<i>S. tuberosum</i>	.	L	L	V	K	A	.	.	.	E	V	N	-	A	A	M	V	L	D	K			
<i>S. oleracea</i>	S	T	L	V	.	.	.	T	.	.	T	.	.	.	E	.	E	-	P	A	M	.	V					
<i>T. aestivum</i>	A	E	-	D	K			
<i>S. × officinarum</i>	K	A	.	A	.	I	E	.	D	T	K	A	M	.	L					
<i>Z. mays</i>	K	A	.	A	.	I	E	.	D	G	K	A	M	.	.					

