***New Phytologist* Supporting Information**

Article title: **Whole-genome duplications followed by tandem-duplications drive diversification of the protein modifier SUMO in Angiosperms**

Authors: Valentin Hammoudi, Georgios Vlachakis, M. Eric Schranz and Harrold A. van den Burg

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The following Supporting Information is available for this article:

Fig. S1 Frequency distribution of the synonymous substitutions per synonymous site (Ks) for the retained ninety gene duplicates in the *AtSUMO1* and *-2* duplication block.

Fig. S2 Brassicaceae *SUMO5* evolved from an ancient *SUMO-like* paralog found in eudicots (see separate Excel file).

Fig. S3 Brassicaceae *SUMO4* originates from an archetype *SUMO* that diversified after the spit of Brassicaceae and Cleomaceae.

Fig. S4 Maximum likelihood (ML) tree of Brassicaceae *SUMO1, -2,* and *-3* genes indicating that *Aethionema arabicum SUMO2* groups sister to the *SUMO2* and *SUMO3* clades.

Fig. S5 Gene tree of the individual UBL domains of DSUL locus in grasses with gene identifiers and bootstrap support values indicated.

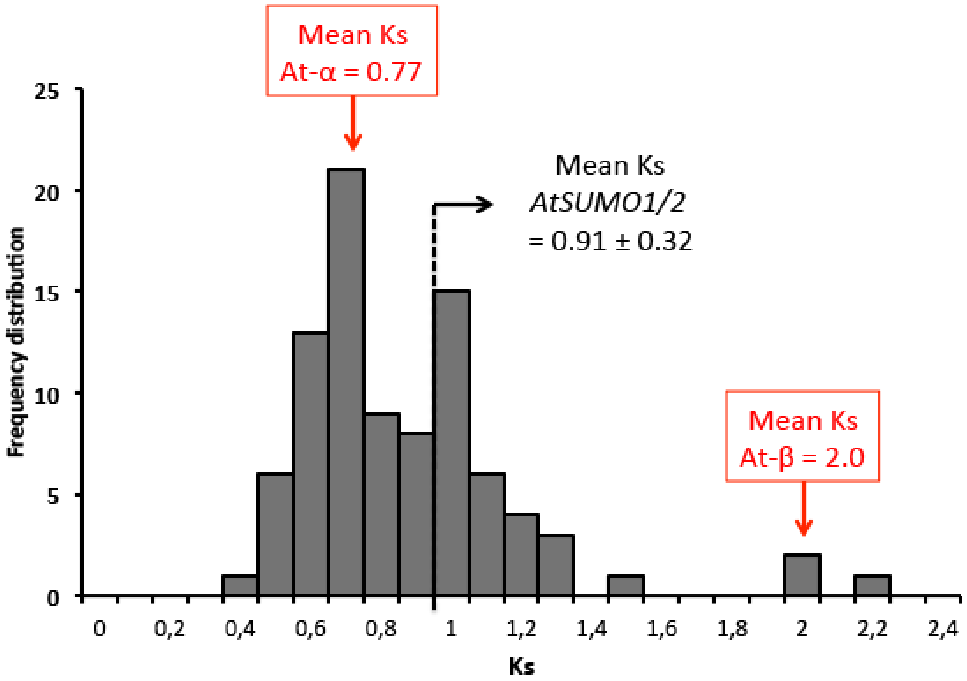
Table S1 List of the different plant genomes used in this study.

Table S2 Annotation of the Brassicaceae SUMO paralogs including expression details.

Table S3 Gene IDs of the here studied dicot *SUMO/SUL* genes in the three different genomic regions: *SUMO*-γA (AtSUMO4), *SUMO*-γB (AtSUMO1/2), and *SUL*-γC (AtSUMO5).

Table S4 Summary of the synteny between *A. lyrata SUMO5* (ID: 16062200; Al4g17860) and the SUMO and SUL genes of the basal eudicots grape vine (*V. vinifera*) and eucalyptus (*E. grandis*).

Fig. S1 Frequency distribution of the synonymous substitutions per synonymous site (Ks) for the retained 90 gene duplicates in the *AtSUMO1* (At4g26840) and -*2* (At5g55160) duplication block. The mean Ks of the *AtSUMO1*<->*AtSUMO2* block is calculated using the 90 gene pairs. For comparison, the mean Ks is given for the At-α and At-β WGDs duplication blocks in *A. thaliana* (Kagale *et al*., 2014). The bin sizes depicted is 0.1 number of substitutions per synonymous site with the first bin ranging from 0 to 0.1 substitutions per synonymous site. De data is obtained from the Plant Genome Duplication Database.



Reference

Kagale S, Robinson SJ, Nixon J, Xiao R, Huebert T, Condie J, Kessler D, Clarke WE, Edger PP, Links MG *et al.* 2014. Polyploid evolution of the Brassicaceae during the Cenozoic era. *Plant Cell* **26**: 2777–2791.

Fig. S2 Brassicaceae *SUMO5* evolved from an ancient *SUMO-like* paralog found in eudicots.
(a) Schematic representation of the gene synteny between the *SUMO5* genomic regions of Arabidopsis (At2g32765), *T. hassleriana* (*Th15853* loci), and eucalyptus (*E. grandis*) *SUL* gene *Eucgr.H00049*. *SUMO5* is syntenic paralog of a divergent *SUL* gene in eucalyptus. Apparently, Arabidopsis *SUMO5* and *Eucgr.H00049* have diversified since their split from their last common ancestor. The genomic position on three different contigs is indicated.
(b) Taxonomic tree with the position of grape, eucalyptus (lineage Myrtales) and the true Rosids (Eurosids), which includes Fabidae (strawberry, soybean) and Malvidae (poplar, Brassicaceae, and *T. hassleriana*).

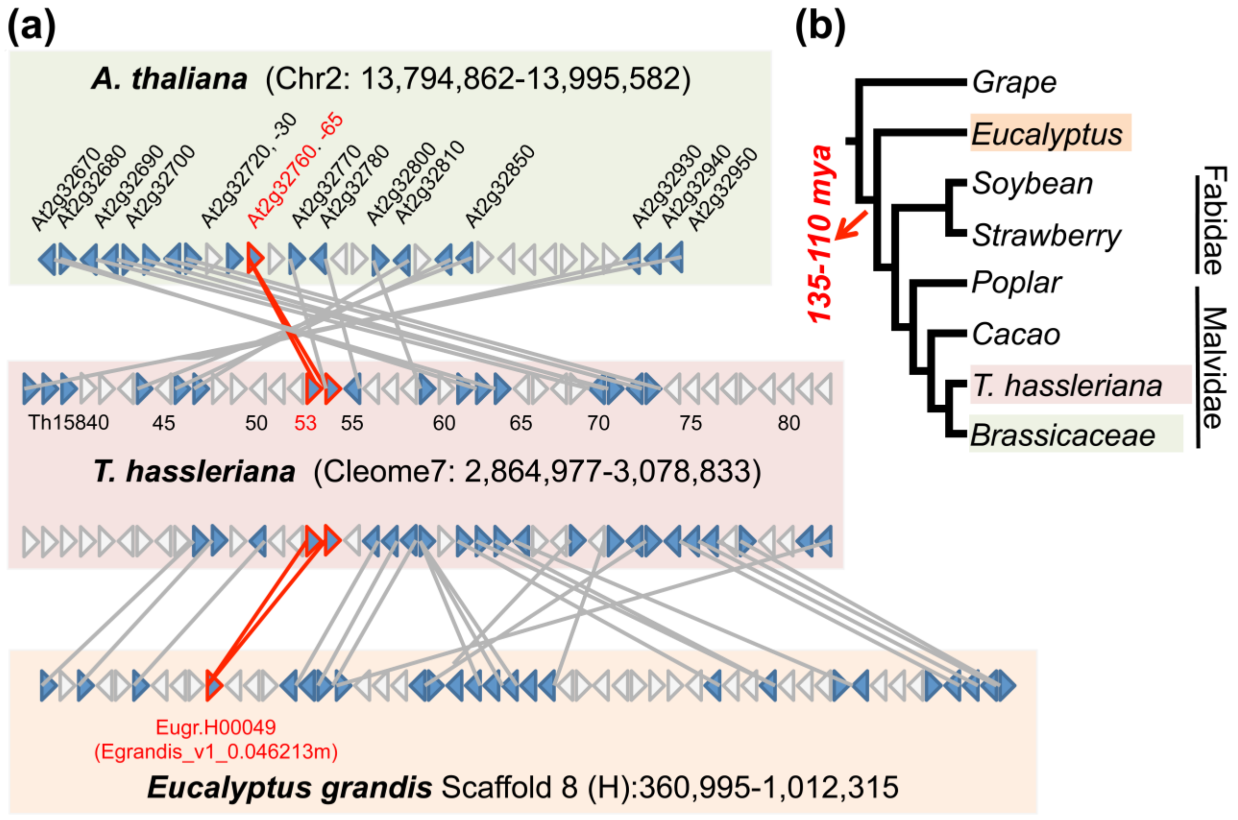


Figure S2

Fig. S3 Brassicaceae *SUMO4* originates from an archetype *SUMO* that diversified after the spit of Brassicaceae and Cleomaceae. Genomic collinearity between the *AtSUMO4* and *-6* (At5g48700, -710) locus in *A. thaliana* and the *Th26484*, *-85*, and *-86* locus in *T. hassleriana* indicates that these five genes represent syntenic paralogues. *Th26486* represents an incomplete tandem duplication of *Th26484* or *-85* genes in the genome assembly, while *Th26484* and *-85* appear to be functional *SUMO* genes.

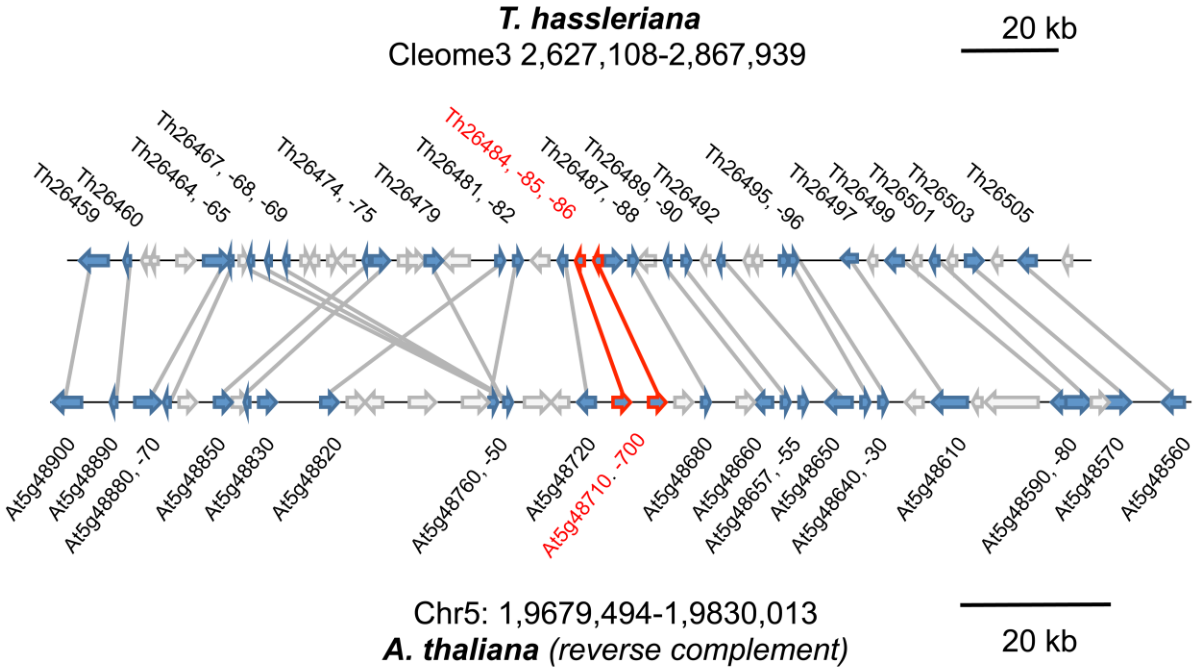


Figure S3

Fig. S4 Maximum likelihood (ML) tree of Brassicaceae *SUMO1*, *-2*, and *-3* genes indicating that *Aethionema arabicum SUMO2* groups sister to the *SUMO2* and *SUMO3* clades. The tandem duplication of *SUMO2::SUMO3* occurred after the split of Aethionema genus. Both *SUMO1*/*2* are conserved at the sequence level (short branch lengths) across Brassicaceae, while *SUMO3* has diversified, been lost, or pseudogenized during the same period. A gene conversion event in *E. salsugineum* has converted *SUMO3* in a *-2* paralog (gene ID Thhalv10015081); which is marked with an asterisks (*). Numbers on the nodes represent bootstrap support values (%) of the individual braches. The scale bar corresponds to the number of nucleotide substitutions per site. The *SUMO1/2* sequences from *T. hassleriana* and *Cleome gynandra* were used as outgroup.

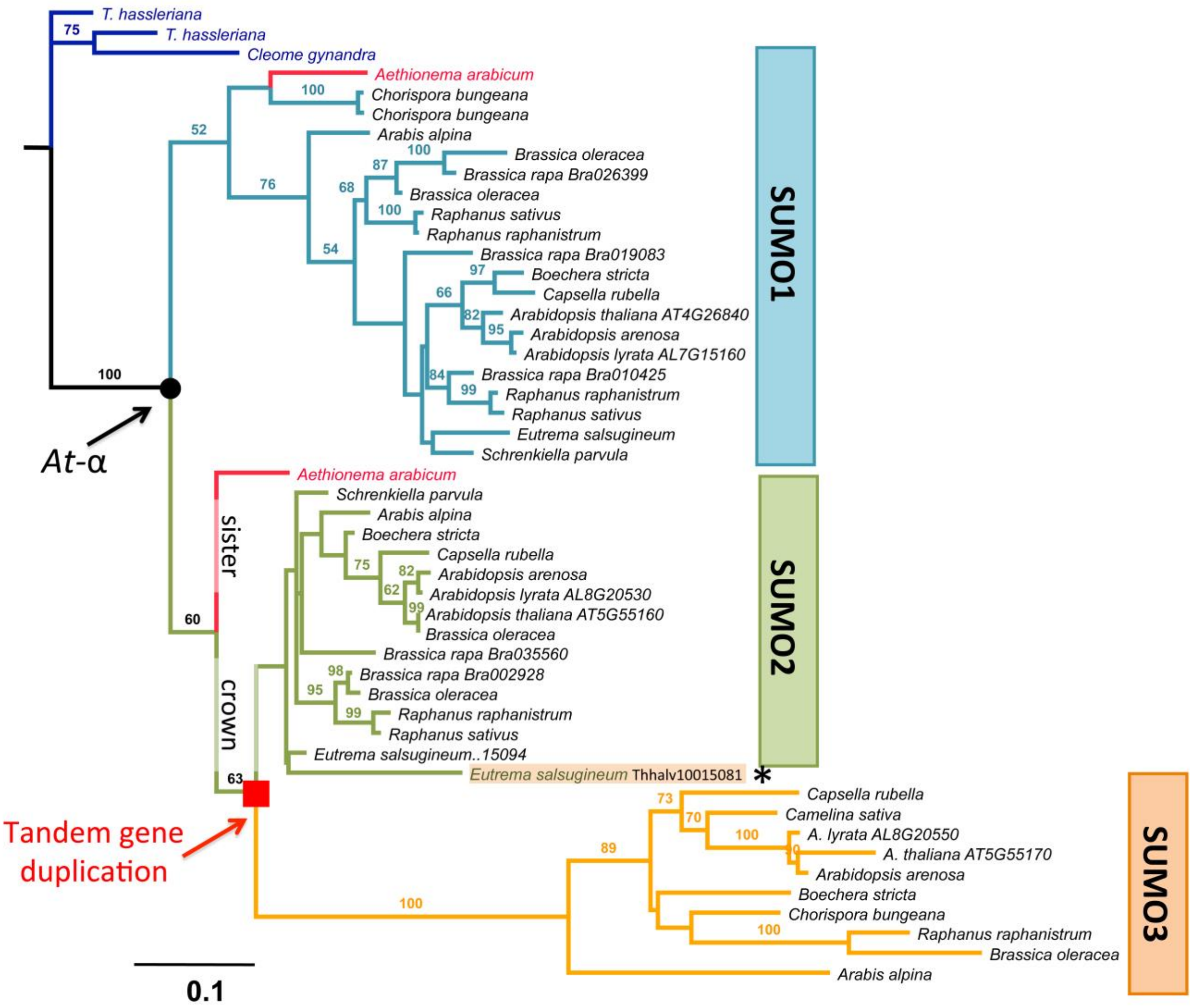


Figure S4

Fig. S5 Gene tree of the individual UBL domains of *DSUL* locus in grasses with gene identifiers and bootstrap support values indicated.

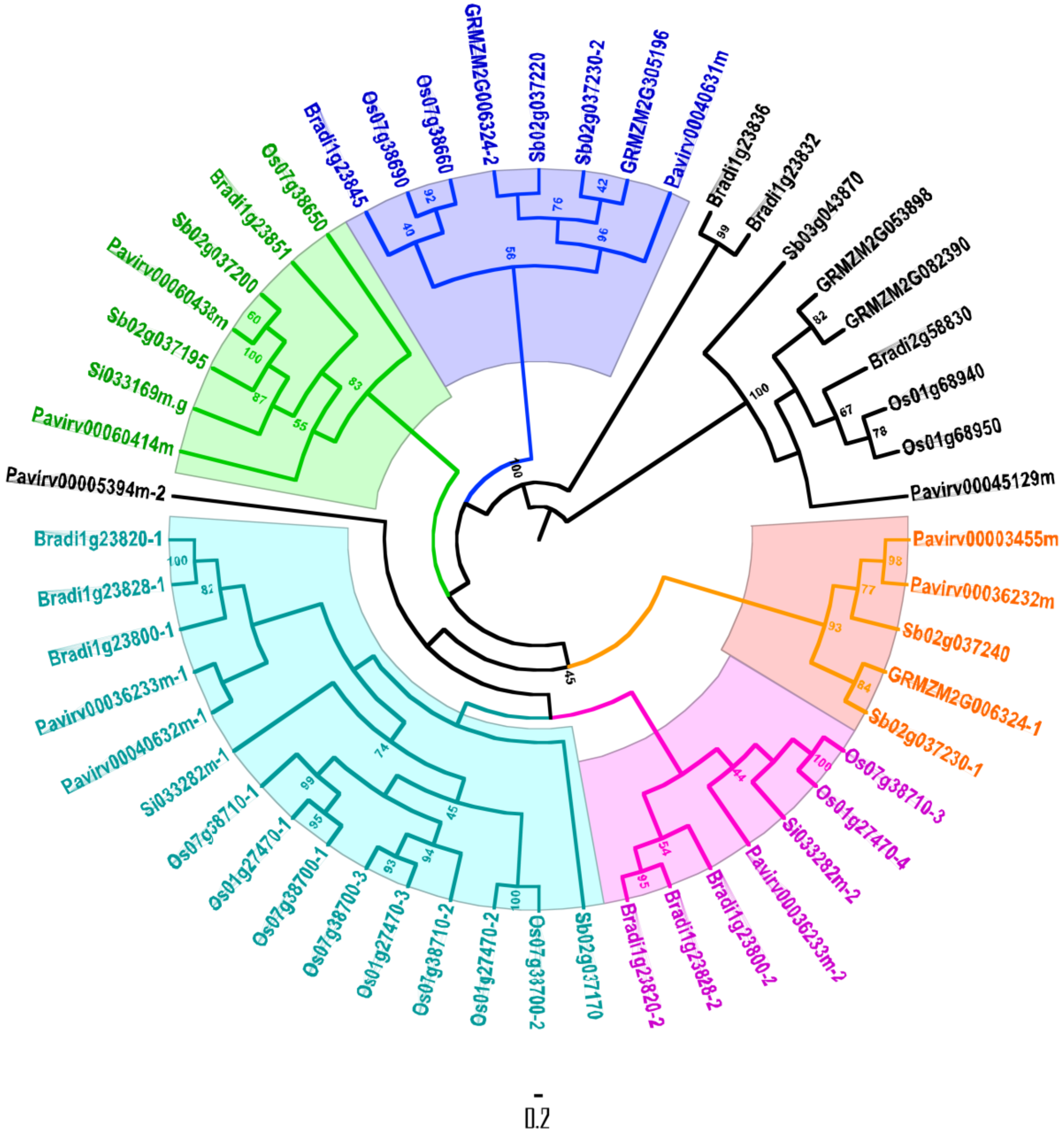


Figure S5

Table S1 List of the different plant genomes used in this study.

Species Name	Common name	Number of Archetype SUMOs	Number of SUMO Paralogs	Genome version	Source	Website	Ref.
Brassicaceae							
Aethionema arabicum	N/A	2 (*)	2	v2.5	CoGE	genomevolution.org/	[1]
Arabidopsis arenosa	Sand Rock-cress	2 (*)	4	-	Luca Comai (UC Davis, USA)	comaiwiki.genomecenter.ucdavis.edu	N/A
Arabidopsis halleri	N/A	2	4	v.1.1	Phytozome 10.1	phytozome.jgi.doe.gov	N/A
Arabidopsis lyrata	Lyrata rockcress	3 (*,#)	5	v1.0	PGDD, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, phytozome.jgi.doe.gov	[2]
Arabidopsis thaliana	Arabidopsis	2 (*,#)	6	TAIR10	TAIR	www.arabidopsis.org	[3]
Arabis alpina	Alpine Rock Cress	2 (*)	3	V4	NCBI	NCBI ID: GCA_000733195.1	[4]
Boechera stricta	Drummond's rockcress	2 (*)	4	v1.2	Phytozome 10.1	phytozome.jgi.doe.gov	N/A
Brassica napus	Rapeseed	N/A	N/A	N/A	JCVI	brassica.jcvi.org/cgi-bin/brassica/gbrowse.cgi	N/A
Brassica oleracea	Cauliflower	5 (*,#)	6	v2.1	BRAD	brassicadb.org,	[5]
Brassica rapa	Turnip	5 (*,#)	7	v1.3	BRAD	brassicadb.org,	[6]
Capsella rubella	Capsella	2 (*,#)	4	v1.0	Phytozome 10.1	phytozome.jgi.doe.gov	[7]
Chorispora bungeana	Blue mustard	N/A (*)	N/A	N/A	NCBI WGS, TSA	NCBI ID: GCA_000715565.1	[8]
Eutrema salsugineum (syn. *Thellungielle halophilla*)	N/A	3 (*)	3	v1.0	Phytozome 10.1	phytozome.jgi.doe.gov	[9]
Leavenworthia alabamica	Rollins Alabama gladecress	N/A	N/A	v0.2	CoGE	genomevolution.org/	[1]
Neslia Paniculata	Ballmustard	N/A	N/A	N/A	CoGE	genomevolution.org/	N/A
Raphanus raphanistrum	Wild radish	N/A (*)	N/A	v1.0	RadishBase (EST)	bin/radish/index.cgi	[10]
Raphanus sativus	Radish	N/A (*)	N/A	v1.0	Raphanus sativus genome database, NCBI (TSA)	bioinfo.bti.cornell.edu/cgi-bin, radish.kazusa.or.jp/blast.html, Genbank: JW988067-JW999999, KA000001-KA089547	[11]
Schrenkiella parvula	N/A	2 (*)	2	v2	CoGE,	genomevolution.org/,	[12]

(syn. *Thellungiella parvula*)					Thellungiella.org	Thellungiella.org/	
Sisymbrium irio	London rocket	N/A	N/A	V2.5	CoGE	genomevolution.org/	[1]
Dicots (non-Brassicaceae)							
Amborella trichopoda	Amborella	1	0	v1.0	AGD	www.amborella.org	[13]
Beta vulgaris	Sugar beat	1 (#)	5	RefBeet-1.1	PGDD	chibba.agtec.uga.edu/duplication/, bvseq.molgen.mpg.de/index.shtml	[14]
Cajanus cajan	Pigeon pea	1 (#)	ND	Nov 2011	PGDD	chibba.agtec.uga.edu/duplication/	[15]
Carica papaya	Papaya	2 (*,#)	0	ASGPBv0.4	PGDD	chibba.agtec.uga.edu/duplication/,	[16]
Cicer arietinum	Chickpea	3 (#)	1	v1.0	PGDD	chibba.agtec.uga.edu/duplication/, legumeinfo.org/	[17]
Citrullus lanatus	Watermelon	2(#)	1	v1.0	PGDD	chibba.agtec.uga.edu/duplication/	[18]
Citrus sinensis	Sweet orange	2 (*,#)	2	v1.1	PGDD, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, phytozome.jgi.doe.gov	[19]
Cucumis sativus	Cucumber	2 (*,#)	1	v1.0	PGDD, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, phytozome.jgi.doe.gov, www.icugi.org/cgi-bin/IcuGI/index.cgi	[20]
Eucalyptus grandis	Eucalyptus, Flooded gum	3 (#)	4	v1.1	PGDD, CoGE, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, genomevolution.org/	[21]
Fragaria vesca	Strawberry	3 (*,#)	3	v1.1	PGDD, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, phytozome.jgi.doe.gov	[22]
Glycine max	Soybean	3(*,#)	3	Wm82.a2.v1	PGDD, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, www.icugi.org/cgi-bin/IcuGI/index.cgi	[23]
Gossypium raimondii	Cotton	6 (*,#)	4	v2.1	PGDD, CoGE	chibba.agtec.uga.edu/duplication/, genomevolution.org/	[24]
Malus domestica	Apple	6 (*)	N/A	v1.0	PGDD	chibba.agtec.uga.edu/duplication/,	[25]
Medicago truncatula	Barrel medic	2 (#)	6	Mt4.0v1	PGDD	chibba.agtec.uga.edu/duplication/	[26]
Nelumbo nucifera	Sacred lotus	2 (#)	2	v1.0	PGDD	chibba.agtec.uga.edu/duplication/, NCBI: PRJNA16800	[27]
Phaseolus vulgaris	Common bean	2 (*,#)	1	v1.0	PGDD	chibba.agtec.uga.edu/duplication/	[28]
Populus trichocarpa	Western poplar	4 (*,#)	0	v3.0	PGDD	chibba.agtec.uga.edu/duplication/	[29]
Prunus mume	Chinese plum	3 (#)	2	v1.0	PGDD	chibba.agtec.uga.edu/duplication/	[30]
Prunus persica	Peach	3 (*,#)	5	v1.0	PGDD	chibba.agtec.uga.edu/duplication/	[31]
Pyrus bretschneideri	Pear	5 (#)	2	v1.0	PGDD	chibba.agtec.uga.edu/duplication/	[32]
Ricinus communis	Castor bean	2 (*,#)	1	v0.1	PGDD	chibba.agtec.uga.edu/duplication/	[33]

Tarenaya hassleriana (syn. Cleome spinosa)	Prickly Spider Flower	5 (*)	2	v4	CoGE	genomevolution.org/	[34]
Theobroma cacao	Cacao	2 (*,#)	3	v1.1	PGDD, CoGE, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, phytozome.jgi.doe.gov	[35]
Vitis vinifera	Grapevine	3 (*,#)	2	Genoscope (Aug 2007)	PGDD, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, phytozome.jgi.doe.gov	[36]
Asterids							
Actinidia chinensis	Kiwifruit	3 (#)	1	May 2013	PGDD	chibba.agtec.uga.edu/duplication/	[37]
Capsicum annuum	Hot pepper	2 (#)	2	v1.55	PGDD	chibba.agtec.uga.edu/duplication/ peppersequence.genomic.cn/	[38]
Mimulus guttatus	Monkey flower	2 (*)	4	V2.0	Phytozome 10.1	phytozome.jgi.doe.gov	[39]
Solanum lycopersicum	Tomato	3 (*,#)	2	v2.4	PGDD, SOL Genomics Network	chibba.agtec.uga.edu/duplication/, solgenomics.net/	[40]
Solanum tuberosum	Potato	2 (*,#)	5	v3.4	PGDD	chibba.agtec.uga.edu/duplication/	[41]
Monocots							
Brachypodium distachyon	Purple false brome	1 (*)	8	v2.1	PGDD	chibba.agtec.uga.edu/duplication/	[42]
Oryza sativa	Rice	2 (*)	6	v7.0	PGDD, Phytozome 10.1	chibba.agtec.uga.edu/duplication/, phytozome.jgi.doe.gov	[43]
Setaria italica	Foxtail millet	ND	ND	v2.1	Phytozome 10.1	phytozome.jgi.doe.gov	[44]
Sorghum bicolor	Sorghum	1 (*)	8	v2.1	PGDD	chibba.agtec.uga.edu/duplication/	[45]
Musa acuminata	Banana	4 (*)	ND	July 2012	PGDD	chibba.agtec.uga.edu/duplication/	[46]
Panicum virgatum	Switchgrass	N/A	N/A	v1.1	Phytozome 10.1	phytozome.jgi.doe.gov	N/A
Zea mays	Maize	2 (*)	4	v6a	PGDD	chibba.agtec.uga.edu/duplication/	[47]
Elaeis guineensis	Oil palm	3	ND	v2.0	PGDD	chibba.agtec.uga.edu/duplication/	[48]

Footnotes:

N/A,	(1) Not or poorly annotated genome assembly limited our estimate of the number of SUMOs/SULs. (2) Publication unknown.
ND,	Not determined.
*,	Archetype SUMO sequences from this species were used to generate the gene tree in Figure 2.
#,	SUMO & SUL sequences from this species are represented in Figure 5, where they form jointly the SUMO-SUL network.

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Table S2 Annotation of the Brassicaceae SUMO paralogs including expression details.
(See the separate Excel file.)

Table S3 Gene IDs of the here studied dicot *SUMO/SUL* genes in the three different genomic regions: SUMO-γA (AtSUMO4), SUMO-γB (AtSUMO1/2), and *SUL*-γC (AtSUMO5). Three ancient SUMO gene clusters are found in the genomes of eudicots. One cluster represents an ancient highly divergent SUMO-like UBL family (*AtSUMO5*), while the other two genomic clusters contain archetype *SUMO* genes (*AtSUMO1*) except for Brassicaceae (*AtSUMO4*). The gene IDs given are obtained from CoGE (*genomevolution.org/*), PGDD (*chibba.agtec.uga.edu/duplication/*), or Phytozome 10 (*phytozome.jgi.doe.gov/*) data repositories. SUL sequences are indicated by a red gene ID.

Species	Taxons				Syntenic homologs		
					SUMOγ–B	SUMOγ–A	*SULγ–C*
Nelumbo nucifera	**Proteales**				*ND*[1]	NNU_022372-RA	
Beta vulgaris	**Caryophyllales**				Bv4_087850_dyxu	*ND*	Bv3_068150_orpx #
Actinidia chinensis	**Asterids**			**Eudicots**	*ND*	Achn127311 Achn307081[2]	Achn266161::Achn266171
Capsicum annuum					CA07g19790	*ND*	CA03g01160 #
Solanum lycopersici					Solyc07g049360.2 Solyc07g064880.2 Solyc12g006010.1[3]	Solyc09g059970.2	Solyc09g091890.2 #
Solanum tuberosum					PGSC0003DMC400038444 PGSC0003DMC400001311 PGSC0003DMC400024516[3]	*ND*	PGSC0003DMC400054530 :: PGSC0003DMC400054531 #
Vitis vinifera	**Vitales**				GSVIVT01030502001	GSVIVT01003301001[4] GSVIVG01003307001[4]	GSVIVT01021058001 #
Eucalyptus grandis	**Myrtales**				Eucgr.K00755::Eucgr.K00756 (Egrandis_v1_0.037737m::Egrandis_v1_0.033394m)	Eucgr.H00789 (Egrandis_v1_0.039699m)	**Eucgr.H00049** # (Egrandis_v1_0.046213m)
Phaseolus vulgaris	**Fabales**	**Fabidae**	**Eurosids**		Phvul.006G047600	Phvul.008G129000	Phvul.003G291000 #
Glycine max					Glyma.08G320500	Glyma.08G350600 Glyma.18G165200	Glyma.05G154000 #, Glyma.08G111700::Glyma.08G111800 #
Fragaria vesca	**Rosales**				mrna01213, mrna03443	mrna17600	mrna06159
Pyrus bretschneideri					Pbr005170.1 Pbr024463.1 Pbr039768.1	Pbr012819.1 Pbr025468.1[2] Pbr033049.1	Pbr030915.1::Pbr030916.1 #
Prunus mume					Pm015148, Pm011903 Pm012555	Pm020340	Pm009507[2]
Prunus persica					ppa012976m, ppa023777m::ppa013753m::ppa013880m ppa023122m	ppa013826m	ppa014098m
Citrullus lanatus	**Cucurbitales**				Cla005336 Cla013517 Cla016281	*ND*	*ND*
Cucumis sativus					16952982::16952983, 16967643, 16982462::16982463	*ND*	*ND*
Ricinus communis	**Malpighiales**	**Malvidae**			29762.m000499	30204.m001744	**29904.m002927 #**
Populus trichocarpa					Potri.002G224700::Potri.002G224800, Potri.014G158300	Potri.014G190300	*ND*
Gossypium raimondii	**Brassicales**				Gorai.001G095200[5] Gorai.001G216800[5] Gorai.009G442500 Gorai.013G158500	Gorai.004G058800	**Gorai.009G349100[4] #**
Theobroma cacao					Thecc1EG002167 (Tc01_g017640)	Thecc1EG002904 (Tc01_g021470)	**Thecc1EG020307 #, Thecc1EG020389::Thecc1EG020390 #** (Tc04_g021070::Tc04_g021080::Tc04_g021100)
Citrus sinensis *Citrus clementina*					orange1.1g034926m Ciclev10017283m	orange1.1g034043m Ciclev10022993m	**orange1.1g048514m::orange1.1g045424m[3] #, Ciclev10006329m[3]**
Carica papaya					evm.TU.supercontig_132.24	evm.TU.supercontig_8.130	*ND*
Tarenaya hassleriana					Th15536 Th11421 Th20205[6]	Th20051, Th26484::Th26485::Th26486 (partial SUM4	**Th15853[7]**

						like)	
Brassicaceae	🟥	🟥	🟫	⬜	**SUMO1** (At4g26840), **SUMO2::SUMO3** (At5g55160:At5g55170)	**SUMO4::SUMO6** (AT5G48700, -710)	**SUMO5** (At2g32765)

1, *ND* indicates that a syntenic paralog was not found in the full genome assembly.
2, Gene fusion or misannotation in the genome assembly.
3, SUMO-like sequences, Solanaceae-specific gene duplication.
4, Not contained in PGDD block.
5, This genomic region appears to correspond to SUMO5 in Arabidopsis, but it resembles the SUMO1 genomic region in Papaya.
6, Potential pseudogene – sequence is incomplete and diglycine motif is absent.
7, Missanotation in the genome assembly, i.e. gene fusion of a *SUMO5* paralog with an upstream gene.
#, Indicates that the diglycine motif is present.
::, Indicates a tandem duplication of SUMO(-like) genes at one locus.

Table S4 Summary of the synteny between *A. lyrata SUMO5* (ID: 16062200; Al4g17860) and the SUMO and SUL genes of the basal eudicots grape vine (*V. vinifera*) and eucalyptus (*E. grandis*).

Species	**Gene ID**		**Number of Anchors**	
	SUMO1 **_SUMO-_γB**	**_SUMO5_** **_SUL-_γC**	**_SUMO1_** **_SUMO-_γB**	**_SUMO5_** **_SUL-_γC**
V. vinifera	GSVIVT01030502001	GSVIVT01021058001	14	30
E. grandis	Eucgr.K00755	Eucgr.H00049	7	12