

## Supplementary Material

**Supplementary Table S1.** Prediction of subcellular localization of actin-related sequences in different plant species.

Species	Prediction	No. of prediction studies per compartment										
		CYT	CSK	NUC	MIT	PLA	PER	ER	GOL	VAC	PM	EXC
<i>Musa acuminata</i>	14	14	0	0	0	0	0	0	0	0	0	0
<i>Hordeum vulgare</i>	1	1	0	0	0	0	0	0	0	0	0	0
<i>Brassica napus</i>	15	15	0	0	0	0	0	0	0	0	0	0
<i>Brassica rapa</i>	6	5	1	0	0	0	0	0	0	0	0	0
<i>Zea mays</i>	39	22	1	3	0	6	0	1	0	0	4	2
<i>Solanum tuberosum</i>	10	9	0	0	1	0	0	0	0	0	0	0
<i>Oryza sativa</i>	2	0	0	0	0	1	0	0	0	0	1	0
<i>Sorghum bicolor</i>	1	1	0	0	0	0	0	0	0	0	0	0
<i>Glycine max</i>	11	3	0	2	3	0	0	3	0	0	0	0
<i>Solanum lycopersicum</i>	5	0	0	3	0	2	0	0	0	0	0	0
<i>Triticum aestivum</i>	3	2	0	0	0	0	0	0	1	0	0	0
<i>Vitis vinifera</i>	2	1	0	0	0	0	0	0	0	0	0	1
Total no. of prediction studies/compartment		73	2	8	4	9	0	4	1	0	5	3

The subcellular localization of proteins highly homologous to *Arabidopsis thaliana* ACT1 (AT2G37620) in other plant species were predicted. The number of distinct prediction studies per species and compartment are shown. The prediction data were obtained from fluorescent protein

(FP), tandem mass spectrometry (MSMS) and related papers in the cropPAL2020 database (cropPAL, <https://croppal.org/>). This platform allows the search for location data across all crop species as well as compares it to Arabidopsis data from SUBA4 (<https://suba.live>). The total number of studies per species, methodology and compartment were determined for reference. CYT, cytosol; CSK, cytoskeleton; ER, endoplasmic reticulum; EXC, extracellular; GOL, Golgi; MIT, mitochondrion; NUC, nucleus; PER, peroxisome; PLA, plastid; PM, plasma membrane; VAC, vacuole.

**Supplementary Table S2.** Prediction of Arabidopsis actin protein subcellular location.

AGI	Predictions	FP	MS/MS	PPI
AT2G37620	mitochondrion	unclear	cytoskeleton	AT2G23420
	cytosol		cytosol	AT2G37620
	cytoskeleton		extracellular	AT3G18780
	nucleus		Golgi (5x)	AT3G53750
			nucleus (2x)	
			plasma membrane (3x)	
			plastid	
			vacuole	
AT3G18780	cytoskeleton	vacuole	cytosol	AT3G46520
	mitochondrion		Golgi (5x)	AT4G29130
	nucleus		mitochondrion	AT4G29130
	cytosol		nucleus (2x)	AT5G59880
			plasma membrane	
			plastid (2x)	
AT3G53750	cytoskeleton	unclear	extracellular	AT2G31200
	mitochondrion		mitochondrion	
	nucleus		nucleus	
	cytosol			
AT5G59370	cytoskeleton	unclear	mitochondrion (2x)	
	mitochondrion			
	nucleus			
	cytosol			
AT5G09810	cytosol	unclear	cytosol	AT2G31200
	cytoskeleton		Golgi (5x)	AT2G37620
	nucleus		mitochondrion (2x)	AT3G12110
	mitochondrion		nucleus (3x)	AT3G18060
			plasma membrane (9x)	AT5G09810
			plastid (3x)	
			vacuole (2x)	
AT1G49240	nucleus	unclear	cytosol (2x)	AT1G72770
	cytoskeleton		extracellular	
	mitochondrion		Golgi (3x)	
	cytosol		nucleus (2x)	
			peroxisome	
			plasma membrane (5x)	
			plastid (4x)	
			vacuole (2x)	

**Supplementary Table S2.** (continued). Prediction of Arabidopsis actin protein subcellular location.

<b>AGI</b>	<b>Predictions</b>	<b>FP</b>	<b>MS/MS</b>	<b>PPI</b>
AT3G12110	nucleus cytoskeleton mitochondrion cytosol	unclear	cytoskeleton Golgi (3x) mitochondrion (2x) nucleus plasma membrane (3x) plastid (2x) vacuole	AT3G18780
AT3G46520	cytoskeleton mitochondrion nucleus cytosol	unclear	mitochondrion nucleus plasma membrane (2x) plastid (3x)	AT2G31200 AT3G18060 AT5G09810

SUBA (<https://suba.live>) provides a subcellular data query platform, protein sequence BLAST alignment, high-confidence subcellular location reference standards, and analytic tools. Concrete data are collected from the query results of actin proteins on the SUBA platform. AGI, Arabidopsis gene identifier; Predictions, Location prediction summary. Any locations predicted by at least one predictor is shown; FP, Localization summary as determined by fluorescent protein assay; PPI, Protein-protein interactions and the SUBAcon location classification of each interacting protein; MS/MS, Localization summary as determined by mass spectrometry. The values in parentheses represent the intensities of the main fragment ions.