

## User's Guide for Plant Reactome Database (Gramene database build # 50)

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**What is Plant Reactome**: The **Plant Reactome** is a free, open-source, curated and peer reviewed pathway database. Our goal is to provide intuitive bioinformatics tools for the visualization, interpretation and analysis of pathway knowledge to support basic research, genome analysis, modeling, systems biology and education.

### Accessing Plant Reactome and important Links

From Gramene: http://www.gramene.org/pathways

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Direct Link: http://plantreactome.gramene.org

**How many plant species are covered in Plant Reactome database :** As of Release # 50, the Plant Reactome contains gene homology-based pathway projections for 62 plant species. We regularly update our database for contents and tools. To see current species and associated statistics see <a href="http://plantreactome.gramene.org/?page\_id=2035">http://plantreactome.gramene.org/?page\_id=2035</a>.

<u>Use case study #1: Tomato:</u> As a researcher you are working on fruit ripening in tomato, and interested in learning about regulation of ripening associated pathways during fruit development. You also have a few related hypotheses that you would like to test, for which you quickly need to extract relevant genes and pathways.

### Q1: Can I identify pathways associated with gene or protein of my interest?



Answer: .....(Fill)

1. Search for a gene or protein in Plant Reactome and find associated pathways: Example: 'CORONATINE-INSENSITIVE1' (or COI1), a gene required for the maternal control of seed maturation, jasmonate-signaled defense response and glandular trichome development (reference: Li et al (2004) : Plant Cell, **16**:126, PMID: ).

Plant Cell. 2004 Jan; 16(1): 126–143. doi: 10.1105/tpc.017954	PMCID: PMC301400
The Tomato Homolog of CORONATINE-INS	NSITIVE1 Is Required for the
Maternal Control of Seed Maturation, Jasm and Glandular Trichome Development <sup>WD</sup>	nate-Signaled Defense Responses, Save Items  Add to Favorites
Gregg A. Howe <sup>1,a,d</sup>	ng Wang, * Mark E. Whaton, * Eran Pichersky,* and
Author information  Article notes  Copyright and License information  This article has been corrected. See Plant Cell. 2004 March: 16(3): 783	The wound response mutant suppressor of prosystemin-mediated responses (spr6) is a weak allele of the [Plant Cell Physiol. 2006]
This article has been <u>cited by</u> other articles in PMC.	Virulence systems of Pseudomonas syringae pv. tomato promote bacterial speck disease in tomato by targeting the jr [Plant J. 2003]
ABSTRACT	Go to: Go to:
Jasmonic acid (JA) is a fatty acid-derived signaling mole responses against herbivores and some microbial pathoge	ale that regulates a broad range of plant defense Jasmonale: an oxylipin signal with many roles in plants. [Vitam Horm. 2005]
established that JA also performs a critical role in anther a other developmental aspects of the plant's life cycle. Here	d pollen development but is not essential for we describe the phenotypic and molecular [Biochimie. 2013]
characterization of a sterile mutant of tomato ( <i>jasmonic a</i> signaling. Although the mutant exhibited reduced pollen maternal control of seed maturation, which was associate	d-braonsitive1 [gal]) that is defective in JA See reviews bability, sterility was caused by a defect in the Bee all with the loss of accumulation of JA-regulated
proteinase inhibitor proteins in reproductive tissues. <i>jail</i> phenotypes, including the inability to express JA-respons	apts exhibited several defense-related Cited by other articles in PMC
defects are caused by the loss of function of the tomato he (COII), an F-box protein that is required for JA-signaled	nolog of CORONATINE-INSENSITIVE1 Control of Carbon Assimilation and Partitioning by Jasmonate: An Accounting of Growth-Defense Tradeoffs [Plants. 1/01]
that the JA/COI1 signaling pathway regulates distinct dev suggest a role for JA in the promotion of glandular tricho	In the second se
INTRODUCTION	Go to: Over the Canon: Within-Plant and Population-Level Heterogeneity in Jasmonate Signaling Engaged by F [Plants. 1/01] Overexpression of SIUPA-like induces cell enlargement, aberrant

Users can type name of a gene in the search box in the Plant reactome home page. Searching for COI1 will give the following result:

(http://plantreactome.gramene.org/content/query?q=coi1&species=Oryza+sativa&species=Entries+without+species&cluster=true)

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About Content Document	ation Tools Community Download Contact coil Search
Search results	s for <i>coi1</i>
Showing 24 of 341	
Species	Set (5 results from a total of 5)
Entries without species (336)      Oryza sativa (5)      Homo sapiens (274)      Aegilops tauschii (3)      Brachypodium distachyon (3)	COIT (Oryza sativa)
	□ <u>NAD(P)+</u>
Hordeum vulgare (3)	□ <u>NAD(P)+</u>
Types	□ <u>NAD(P)H</u>
<ul> <li>Chemical Compound (244)</li> <li>Regulation (82)</li> <li>OtherEntity (6)</li> </ul>	□ <u>NAD(P)H</u>
<ul> <li>Set (5)</li> <li>Reaction (3)</li> </ul>	Complex (1 results from a total of 1)
Complex (1)	COl1 bound to JA-Ile (Oryza sativa)
Compartments	COI1 a Fbox protein act as the JA receptor and binds to JA-IIe conjugate
<ul> <li>cytosol (198)</li> <li>mitochondrial matrix (41)</li> </ul>	Reaction (3 results from a total of 3)
Golgi lumen (8)	••• COI1 binds JA-Ile conjugate (Oryza sativa)
nucleoplasm (2)	COI1 a Fbox protein act as the JA receptor and binds to JA-Ile conjugate
plastid stroma (2)	Here Wiguitination and proteosomal degradation of JAZ (Oryza sativa)





The results also suggest that the COI1 gene product participates in a reaction where it binds Jasmonate-IIe and forms a complex. Now click on the Reaction Link for 'COI1



binds JA-Ile conjugate (Solanum lycopersicum)'. It will take you to the Reaction detail page which tells you about how the reaction was curated/projected, inputs and outputs of the reactions and the pathway this reaction is associated with.

Click on the '+' sign next to the 'Plant Patwhays (Solanum lycopersicum).

The hierarchy chart opens up telling about the pathways this reaction is associated with. In this case the Jasmoic acid signaling.

http://plantreactome.gramene.org/content/detail/8973392

Click on the hyperlinked reaction name and it will lead you to the **<u>Pathway Browser</u>** highlighting the selected reaction

http://plantreactome.gramene.org/PathwayBrowser/#SPECIES=8969285&DIAGRAM=8





### 973852&ID=8973392&PATH=8973668,8973667,8973680,



In the browser you see the reaction and pathways highlighted to suggest its placement in the data structure.

Zoom-in/out functions for pathway viewer are in the red-box

Download the pathway in different formats (click the download button in grey box) See list of molecules participating in the reaction (click the molecules button in cyan

box). You will see the highlighted genes products and inputs/outputs Click on the + sian next to chemical compounds of genes based on your interest to see a full list. Depending on your selection in the left hand tree, if it is for pathway you will see a larger list. Also there is a download data option in this tab.





We chose to use gene products section to look for the tomato gene that was mapped to this reaction. It turns out there is one tomato gene, Solyc05g052620.2 was mapped to COI1 function in this pathway.



#### \*\*\*\*\*

#### Q2. Can I find a similar event in Potato? And find COI1 gene homologs.

A. Fill in your answer.....

#### Navigating Projected pathways in another plant species:

On the Pathway Browse page, select 'Solanum tuberosum' from the species drop down list (see red box)





# As soon as you select the potato species, the browser view will refresh.

#### http://plantreactome.gramene.org/PathwayBrowser/#SPECIES=8973856&DIAGRAM=8 978286&PAT=8978105,8978104,8978117&





# Result: You will see that this reaction was not projected for potato, as JA-lle to JAZ connection is missing in potato.

There are following possible reasons for this difference.

The potato gene homolog was not identified that's why we do not see the mapped gene and the missing reaction from potato.

Absence of potato gene could be due to

- Annotated gene was not identified by the potato genome project. Therefore missing
- The gene was there but it didn't meet the homolog calling criteria, thus missing from the gene family cluster
- This may be case of either true biology or evolutionary effects or the genotype that was sequenced to produce reference potato genome.
- These are all good observations, that become research questions that can be discussed in the Genome exercise.

### Projections in other species:

Let's try another species 'Glycine max', strawberry and peanut. We see the reaction being projected in all three species.

Link for 'Glycine Max':

http://plantreactome.gramene.org/PathwayBrowser/#SPECIES=8962006&DIAGRAM=8 969281&ID=8968635&PATH=8969100,8969099,8969112&DTAB=MT





### Link for peanut (Arachis ipaensis):

http://plantreactome.gramene.org/PathwayBrowser/#SPECIES=8928096&DIAGRAM=8932127&ID=8931817&PATH =8931939,8931938,8931951&DTAB=MT



### Link for strawberry (Fragaria vesca)

http://plantreactome.gramene.org/PathwayBrowser/#SPECIES=8900291&DIAGRAM=8904569&ID=8904260&PATH =8904377,8904376,8904389&DTAB=MT





Q3. Can I compare nplex 🔵 in 🦲 S Analysis Tool: a pathway the analysis between reference Species Comparis species rice rm the corr and anyone of of 147 the 62 plant species for which pathway projection are available?

Example: jasmonic acid

signaling pathway comparisons between tomato and rice

# Step 1: navigate and open Jasmonic acid signaling pathways in reference species rice (*Oryza Sativa*). Link:

http://plantreactome.gramene.org/PathwayBrowser/#DIAGRAM=6787011&PA TH=2894885,2744345,2744341&DTAB=AN&ANALYSIS=MjAxNjA1MTcxNDM zMTFfNDE%2525253D





Step 3: From the species comparison drop down option select your favorite species and then hit compare.

# As a result, a new pathway browsing window, showing the projection of Tomato pathway over the reference pathway from rice will open as shown here.



In this pathway diagram, complete blue boxes represent rice enzyme and genes for which no corresponding orthologs were found in tomato. The filled yellow box represents the enzyme for which tomato ortholog(s) have been found. The box filled with yellow and blue represent the difference in the number of genes mapped to this particular enzyme in reference species rice and the projected species tomato. Users can click on a given enzyme to see for which rice genes homologs have been identified in the tomato (rice gene highlighted in yellow color) and for which gene orthologs were not found in tomato (genes highlighted with blue color.

# Q4. How to link to other external DBLinks for accessing Genome Browser, gene expression, etc. from Plant Reactome

We currently provide external links for Arabidopsis, rice and Maize only. We are working on tomato and other species.



### Q5. How one can we analyze transcriptomic data using Plant Reactome?

A note about web browsers: At present the Firefox, Netscape and Safari are having problems with projection of expression data on plant pathways. Although Safari, allows the display of the expression data mapped to pathways in tabulated format. We suggest users currently use Chrome.

Here is the case study for analysis of transcriptomic data analysis of a publicly available data from published study.

Study: Transcriptome analysis of various tissues in tomato cultivar Heinz

Reference: PMID: 22660326 (<u>Nature.</u> 2012 May 30;485(7400):635-41. doi: 10.1038/nature11119). The tomato genome sequence provides insights into fleshy fruit evolution.

Data: http://ted.bti.cornell.edu/cgi-bin/TFGD/digital/experiment.cgi?ID=D004

File: https://www.dropbox.com/s/0i3b1jqoroxb9gz/tomato-baseline-ted-bti-D004-3-log2fold.txt?dl=0

**Experiment description:** Illumina RNA-seq analysis of leaves, roots, flower buds, fully opened flowers, and 1 cm, 2 cm, 3 cm, mature green, breaker, and breaker+10 fruits of tomato cultivar Heinz, and leaves, immature green, breaker, and breaker+5 fruits of *Solanum pimpinellifolium* 

1. Go to the pathway browser page of your favorite species, for example, tomato

http://plantreactome.gramene.org/PathwayBrowser/#SPECIES=8969285&DIAGRA M=8973667&PATH=8973668

 Click on Tools-→ Choose a data file to upload (under Analysis Tools) window or enter your data into the text box (as shown in the following figure)

Event Hierarchy:		
Plant pathways	Analysis Tools	
Metabolism and regulation	This tool merces pathway identifier mapping, overrepresentation and expression analysis into a single tabbed data analysis	portal, with integrated visualization and
Generation of precursor metal:	summary features.	
Amino acid metabolism	Select a file from your computer and click on the "Analyse" button to perform the analysis.	
Amine and polyamine biosynth		
Carbohydrate metabolism	Select data file for analysis Choose File no file selected Project to Oryza sativa	Analyse
Cofactor biosyntheses	Click here to naste your data or try example data sets	
Hormone biosynthesis and sig		
AA biosynthesis I	Paste the data to analyse	Some examples:
IAA biosynthesis II	#probeset Bud-vs-leaf flower-vs-leaf root-vs-leaf fruit-1cm-vs-leaf fruit-2cm-vs-leaf fruit-3cm-vs-leaf solu-01-00200000 2.0.2022005167.0.240050266.0.20200666477.0.261272410.0.0202666142.0.201000550	Uniprot accession list
IAA biosynthesis VI (via indentified)	Solyc01g005010.2 -0.590218226 -0.503374966 -0.053439259 -0.053439259 -0.186413124 -0.569309236	Gene identifier list
Head Auxin transport	Solyc01g005020.2 0.165728067 0.443918619 1.433483167 0.860255259 1.346238502 1.543916343 Solyc01g005030.2 0.134899519 -0.008441774 0.742677915 0.128753844 0.283372048 0.909693579	Microarray data
IAA conjugate biosynthesis	Solyc01g005040.2 -1.150559677 -0.948925815 -1.039528364 -1.026231542 -1.150559677 -0.783188611 Solyc01e005050 2 6 201241771 5 406332578 0 201633861 0 0 23878686 0 189033824	
B      Brassinosteroid biosynthesis	Solyc01g005060.2 0.696721962 0.820321341 0.142856758 3.017988119 1.82789456 0.820321341	Metabolomics data
trans-zeatin biosynthesis	Solyc01g005010.2 2.23534641 4.043566698 0.094859186 0.9922/1572 0.379588663 0.054966459 Solyc01g005080.2 0.789439758 2.037050976 1.080336207 0.579918107 0.097646692 -0.587324864	
Jasmonic acid biosynthesis	Solyc01g005090.2 0 0 4.434294618 0.070389328 0 0 Solyc01g005100.2 -0.938083808 -0.821694743 -0.184612125 -0.600170735 -0.735590944 -0.819265963	
Jasmonic acid signaling	Solyc01g005110.2 1.814761393 1.180538272 1.046597589 0.741348235 -0.254512035 0.083039585 Solyc01g005100 2.2.46808493 -4.827098877 2.131705679 1.108753852 1.168745847 -0.20107010	
GA12 biosynthesis	Solyc01g005130.2 -1.911136013 -1.469203287 0.439800529 -1.5340836 -1.556824744 -0.011502971	
gibberellin biosynthesis I (n.	Glear Project to Oniza estiva	Analyse
🙂 🐝 gibberellin biosynthesis II (e		
🙂 🐝 gibberellin biosynthesis III (	Anadas Utilization Provide Pro	
🗉 🐝 ABA biosynthesis and medi	Overview Molecules Expression Analysis Processes Dowinoads	
Ethylene biosynthesis and s	* Metabolism and regulation Species: Solanum lycopersicum	
Strigolactone Biosynthesis	Summation	
Strigolactone signaling		
🗉 🐳 Auxin signalling	This event has been computationally inferred from an event that has been demonstrated in another species.	
Grassinosteroid signaling	The inference is based on Ensembl Compara orthology projection. Briefly, reactions for which all involved PhysicalEntities (in inp	ut, output and catalyst) have a
Salicylic acid signaling	mapped ortholog or paralog are inferred to the other species. High-level events are also inferred for these events to allow for eas	ier navigation.
· · · · · Secondary metabolite biosynth	Details of projection methods and parameters may be found here.	
* Fatty acid and lipid metabolism		



3. After uploading/ pasting the data, click on analyze to map gene expression data on Tomato. Users also have an option to project Tomato expression data on the reference rice pathways by checking the small icon "Project to Oryza sativa". Users can browse pathways belonging to a category (e.g. Carbohydrate metabolism) or individual pathways or sub-pathways by selecting the options from left-hand side window that list pathways



- 4. The Figure above shows all the pathways involved in the carbohydrate metabolism that show differential expression between fruit and leaf tissue. The Bottom right-hand side bottom panel lists the pathways and associated data.
- 5. Users can browse this list or the left-hand side panel to select an individual pathway, such as Chorismate biosynthesis pathway to see the expression of genes mapped to a pathway. At present we are having problem to do that directly on the tomato and some of the projected species. So users can select reference species rice and can project the tomato expression data on rice pathways.



6. By clicking on a given enzyme, users can open a pop-up window showing the rice genes and their tomato orthologs with expression data. As shown in the figure.

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← → C 🗋 plantreactome.gran	nene.org/PathwayBrow	ser/#DIAGRAM=1119430	0&ID=1121887&DTAB=AN&	ANALYSIS=	MjAxNjA1	LMDMxN;	15MjhfMjI%	253D					☆ (0) =
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Pathways for: Oryza	ı sativa		Tour this pathway brow	vser? Hide						Pro	tein 🔵 Sm	all molecule 🔵	Complex 🔵 🔻
Event Hierarchy:													
chorismate biosynthesis (9/16) FDR: 18		eaction/Entity:	▲ ▼ Highlight	All X									<del>-</del>
glycine betaine biosynthesis III (1/3) FDF													12.59
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alvin cycle (22/28) FDR: 1E0		3040	_										
blichyl-diphosphooligosaccharide biosynt	(120		<u> </u>										
achyose biosynthesis (1/4) FDR: 1E0	(1178	(dehudro shikimate)	Components for Shikimal	te kinase:									
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shalose degradation II (3/4) FDR: 1E0	folate polyglutamylation		Oryza sativa	7	12	0.008	1E0	1E0	5	5	0.007	0.36	0.434
ylan Biosynthesis (4/6) FDR: 1E0	IAA biosynthesis II		Oryza sativa	4	7	0.005	1E0	1E0	2	2	0.003	0.698	0.189
yloglucan biosynthesis (4/16) FDR: 1E0	glycine biosynthesis I		Oryza sativa	4	7	0.005	1E0	1E0	1	1	0.001	0.893	0.71
logalacturonan biosynthesis (1/2) FDR: 1	chorismate biosynthesis		Oryza sativa	2	16	0.011	1E0	1E0	7	7	0.01	0.659	0.813
ctor biosyntheses (82/154) FDR: 1E0	phenylpropanoid biosynt	hesis	Oryza sativa	13	26	0.017	1E0	1E0	14	14	0.02	0.59	0.132
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A biosynthesis I (11/32) FDR: 1E0	Result Mapping		,			H 🖲 6	1-80 of 179 🕕	• •					

7. The expression data mapped to the various pathways in plant Reactome can also be downloaded.

# Q6. Can I download Plant Reactome database for conducting analysis in my local desktop.

Plant Reactome is a complex platform and currently we do not provide this option.

### Q7. How can I send my feedback to Plant Reactome group:

If you have questions or comments about Plant Reactome data, our website, or the downloads that we provide, we would be pleased to hear from you!

You can email us at reactome-curator@gramene.org