



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>

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 http://plantreactome.gramene.org/?page_id=2035.

Use case study #1: Tomato: 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:).

The screenshot shows a PubMed entry for a 2004 article in Plant Cell. The title is "The Tomato Homolog of CORONATINE-INSENSITIVE1 Is Required for the Maternal Control of Seed Maturation, Jasmonate-Signaled Defense Responses, and Glandular Trichome Development". The authors listed are Lei Li, Youfu Zhao, Bonnie C. McCaig, Byron A. Wingerd, Jihong Wang, Mark E. Whalon, Eran Pichersky, and Gregg A. Howe. The abstract describes the role of the JA signaling pathway in Arabidopsis and its homolog in tomato. The article has been cited by other articles in PMC.

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>)

The screenshot shows the Plant Reactome search results for the query 'coi1'. The search bar at the top shows 'coi1' and a search button. Below the search bar, the results are categorized into Species, Types, and Compartments. Under Species, there are 5 results from a total of 5, including COI1 (Oryza sativa), NAD(P)+, and NAD(P)H. Under Types, there are 1 result from a total of 1, which is COI1 bound to JA-Ile (Oryza sativa). Under Compartments, there are 3 results from a total of 3, including COI1 binds JA-Ile conjugate (Oryza sativa) and Ubiquitination and proteosomal degradation of JAZ (Oryza sativa).

By default the database searches are on '*Oryza sativa*'. However, from the Species section, uncheck the *O. sativa* and entries without species and click on the 'more' option to find tomato (*Solanum lycopersicum*). After the selection you will see

The screenshot shows the REACTOME website interface. The search results for 'COI1 binds JA-Ile conjugate' in *Solanum lycopersicum* are displayed. The page includes a navigation menu, a search bar, and a detailed view of the reaction. The reaction is summarized as 'COI1 binds JA-Ile conjugate' in *Solanum lycopersicum*. The summary notes that this event is computationally inferred from an event demonstrated in another species. The inference is based on Ensembl Compara orthology projection. The page also lists locations in the PathwayBrowser, components of the entry (input and output entries), and inferred entries (inferred from and orthologous events).

<http://plantreactome.gramene.org/content/query?q=coi1&species=Solanum+lycopersicum&cluster=true>

The screenshot shows the REACTOME website search results for 'coi1'. The search results are displayed under the heading 'Search results for coi1'. The results show 2 items. The first item is 'Species' with a list of species: *Solanum lycopersicum* (2), 'Entries without species' (336), *Homo sapiens* (274), *Oryza sativa* (5), *Aegilops tauschii* (3), and *Brachypodium distachyon* (3). The second item is 'Reaction' (1 result from a total of 1) for 'COI1 binds JA-Ile conjugate (Solanum lycopersicum)'. The reaction is summarized as 'COI1 binds JA-Ile conjugate (Solanum lycopersicum)'. The summary notes that this event is computationally inferred from an event demonstrated in another species. The third item is 'Complex' (1 result from a total of 1) for 'COI1 bound to JA-Ile (name copied from entity in Oryza sativa) (Solanum lycopersicum)'. The complex/polymer is summarized as 'COI1 bound to JA-Ile (name copied from entity in Oryza sativa) (Solanum lycopersicum)'. The summary notes that this complex/polymer has been computationally inferred (based on Ensembl Compara orthology projection).

The results also suggest that the COI1 gene product participates in a reaction where it binds Jasmonate-Ile 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 **Pathway Browser** highlighting the selected reaction

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

The screenshot shows the REACTOME website interface. At the top, there is a navigation bar with links for 'About', 'Content', 'Documentation', 'Tools', 'Community', 'Download', and 'Contact'. A search bar is located on the right side of the navigation bar. Below the navigation bar, the main content area displays the reaction name 'COI1 binds JA-Ile conjugate' and the species 'Solanum lycopersicum'. Underneath, there is a section titled 'Summation' which contains text explaining that the event has been computationally inferred from another species. Below this, there is a section titled 'Locations in the PathwayBrowser' which shows a hierarchical tree structure of pathways, with the selected reaction highlighted in purple.

me.gramene.org/content/detail/8973392

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REACTOME

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e.g. O95631, NTN1, signaling b Search

COI1 binds JA-Ile conjugate

Species Solanum lycopersicum

Summation

This event has been computationally inferred from an event that has been demonstrated in another species.

The inference is based on Ensembl Compara orthology projection. Briefly, reactions for which all involved PhysicalEntities (in input, output and catalyst) have a mapped ortholog or paralog are inferred to the other species. High-level events are also inferred for these events to allow for easier navigation.

Details of projection methods and parameters may be found [here](#).

Locations in the PathwayBrowser

- Plant pathways(Solanum lycopersicum)
 - Plant pathways(Solanum lycopersicum)
 - Metabolism and regulation(Solanum lycopersicum)
 - Hormone biosynthesis and signaling(Solanum lycopersicum)
 - Jasmonic acid signaling(Solanum lycopersicum)
 - COI1 binds JA-Ile conjugate(Solanum lycopersicum)

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 + sign 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.

The screenshot displays the Gramene Pathway Browser interface. On the left, the 'Event Hierarchy' shows the pathway structure, with 'COI1 binds JA-Ile conjugate' selected under 'Jasmonic acid signaling'. The central diagram illustrates the reaction where COI1 binds to JA-Ile, catalyzed by Solyc05g052620.2. The right-hand panel shows the 'Molecules (2/42)' tab, listing chemical compounds and proteins. The protein list includes Solyc05g052620.2 (Ensembl:Solyc05g052620.2 (2x)), Solyc01g006650.1 (Ensembl:Solyc01g006650.1 (1x)), and Solyc01g014480.2 (Ensembl:Solyc01g014480.2 (1x)).

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)

Pathways for: **Solanum lycopersicum**

Event Hierarchy:

- Plant pathways
 - Metabolism and regulation
 - Generation of precursor metabolites and energy
 - Amino acid metabolism
 - Amine and polyamine biosynthesis
 - Carbohydrate metabolism
 - Cofactor biosyntheses
 - Hormone biosynthesis
 - IAA biosynthesis I
 - IAA biosynthesis II
 - IAA biosynthesis VI
 - Polar Auxin transport
 - IAA conjugate biosynthesis I
 - brassinosteroid biosynthesis II
 - trans-zeatin biosynthesis
 - jasmonic acid biosynthesis
 - Jasmonate conjugation
 - COI1 binds JA-ile conjugate**
 - Nuclear uptake of JAZ
 - Ubiquitination and proteosomal degradation
 - JAZ binds MYC like
 - JAZ/MYC binds NINJA
 - MYC induced transcription of JA inducible genes
 - JAZ/MYC/NINJA binds TPL

Find Reaction/Entity: Highlight All X

Protein Small molecule Complex

Overview Molecules (2/42) Expression Analysis Processes Downloads

COI1 binds JA-ile conjugate Species: **Solanum lycopersicum** Download Info

Proteins (1/36)

Gene ID	Ensembl ID	Count
Solyc05g052620.2	Ensembl:Solyc05g052620.2	(2x)
Solyc01g006650.1	Ensembl:Solyc01g006650.1	(1x)
Solyc01g014480.2	Ensembl:Solyc01g014480.2	(1x)
Solyc01g095580.2	Ensembl:Solyc01g095580.2	(1x)

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

<http://plantreactome.gramene.org/PathwayBrowser/#SPECIES=8973856&DIAGRAM=8978286&PAT=8978105,8978104,8978117&>

Pathways for: **Solanum tuberosum**

Event Hierarchy:

- Plant pathways
 - Metabolism and regulation
 - Generation of precursor metabolites and energy
 - Amino acid metabolism
 - Amine and polyamine biosynthesis
 - Carbohydrate metabolism
 - Cofactor biosyntheses
 - Hormone biosynthesis and signaling
 - IAA biosynthesis I
 - IAA biosynthesis II
 - IAA biosynthesis VI (via indole-3-acetamide)
 - Polar Auxin transport
 - IAA conjugate biosynthesis I
 - brassinosteroid biosynthesis II
 - trans-zeatin biosynthesis
 - jasmonic acid biosynthesis
 - Jasmonic acid signaling
 - GA12 biosynthesis

Find Reaction/Entity: Highlight All X

Protein Small molecule Complex

Overview Molecules (36) Expression Analysis Processes Downloads

Jasmonic acid signaling Species: **Solanum tuberosum** Download Info

Result: You will see that this reaction was not projected for potato, as JA-Ile 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=8969281&ID=8968635&PATH=8969100,8969099,8969112&DTAB=MT>

The screenshot shows the Gramene Pathway Browser interface for the species 'Glycine max'. The main pathway diagram illustrates the conversion of JA-Ile to JAZ. Key components include:

- Enzymes:** Jasmonyl-isoleucine synthetase (green box) and COI1 bound to JA-Ile (yellow box).
- Reactants:** L-Ile, ATP, and PPI.
- Products:** Jasmonyl-isoleucine and AMP.
- Annotations:** 'Homologues of OS1g0853400' is noted near the COI1 bound to JA-Ile complex.
- Event Hierarchy (Left Panel):**
 - Cofactor biosyntheses
 - Hormone biosynthesis and signaling
 - IAA biosynthesis I
 - IAA biosynthesis II
 - IAA biosynthesis VI (via indole-3-acetamide)
 - Polar Auxin transport
 - IAA conjugate biosynthesis I
 - brassinosteroid biosynthesis II
 - trans-zeatin biosynthesis
 - jasmonic acid biosynthesis
 - Jasmonic acid signaling
 - Jasmonate conjugation
 - COI1 binds JA-Ile conjugate
 - Nuclear uptake of JAZ
 - Ubiquitination and proteasomal degradation
 - JAZ binds MYC like
 - JAZ/MYC binds NINJA
 - MYC induced transcription of JA inducible genes

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>

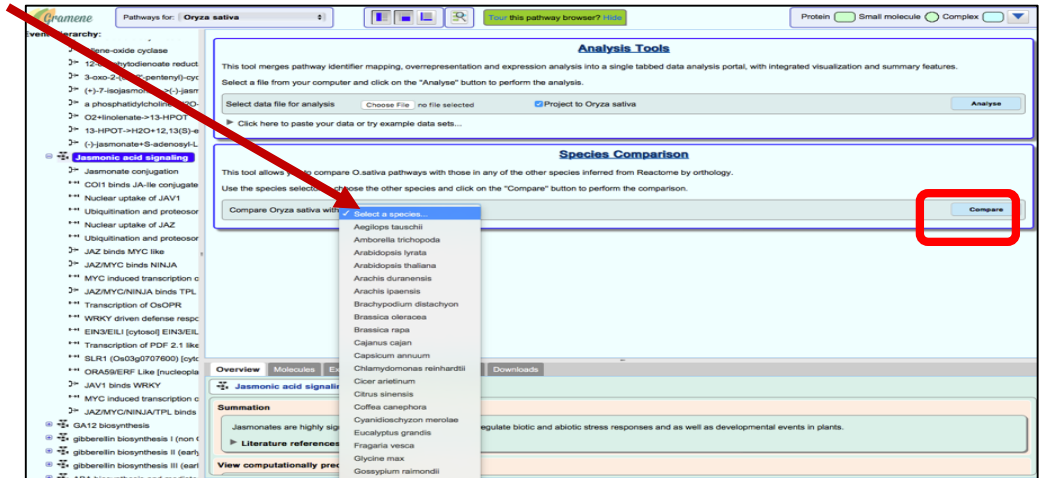
Q3. Can I compare a pathway between reference species rice and anyone of the 62 plant species for which pathway projection are available?

Example: jasmonic acid

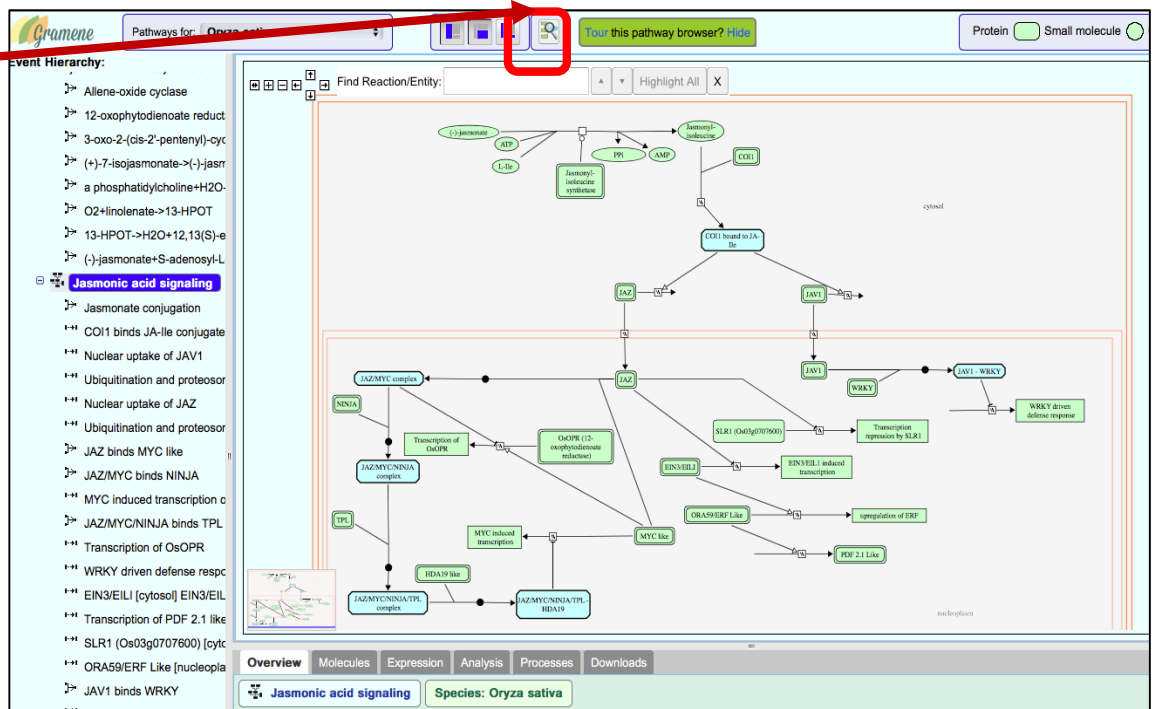
signaling pathway comparisons between tomato and rice

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

<http://plantreactome.gramene.org/PathwayBrowser/#DIAGRAM=6787011&PATH=2894885,2744345,2744341&DTAB=AN&ANALYSIS=MjAxNjA1MTcxNDMzMTEfNDE%2525253D>



Step2: Click on the Tools (boxed in the figure). A new window with Tools menu opens as following.



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

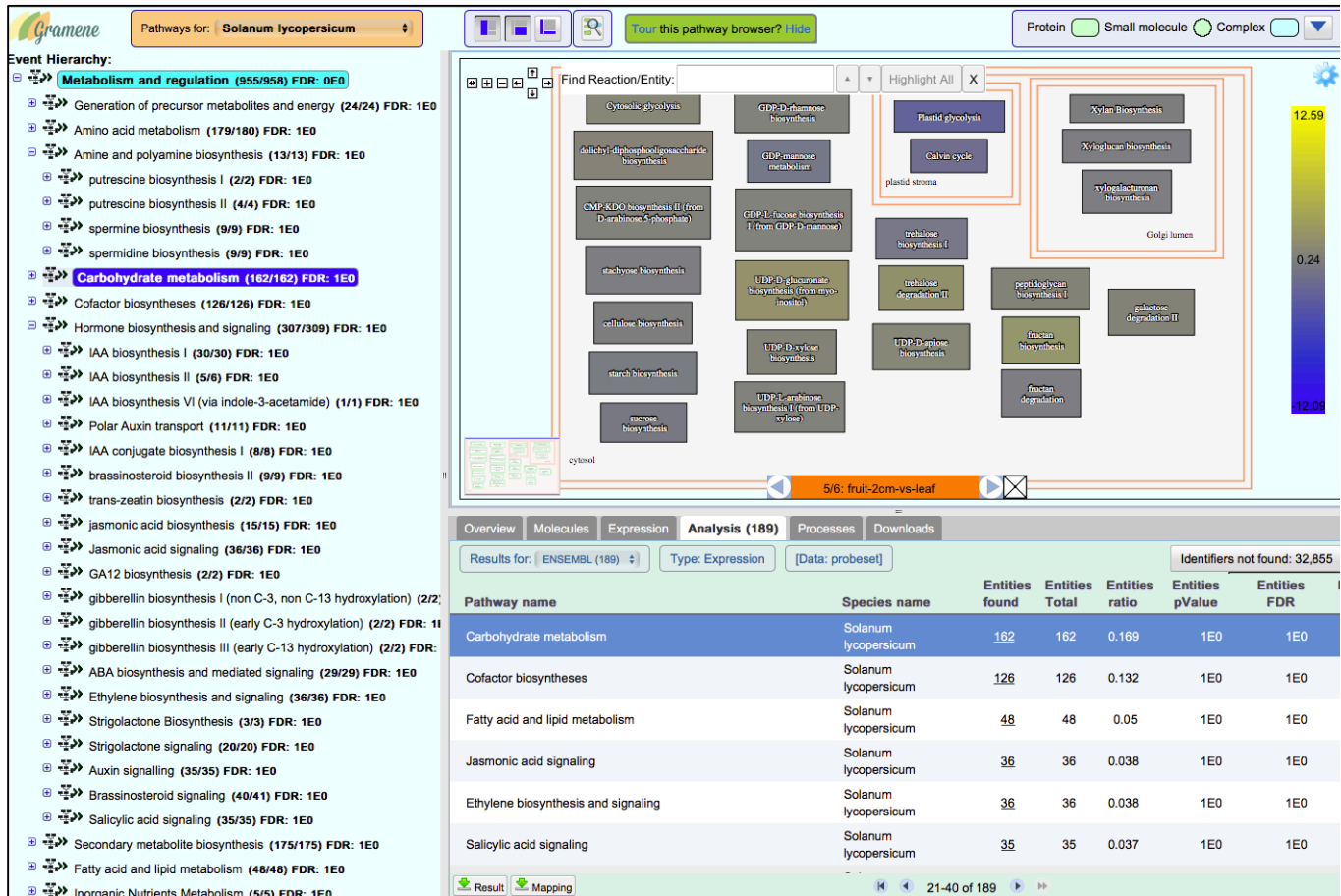
As a result, a new pathway browsing window, showing the projection of Tomato pathway over the reference pathway from rice will open as shown here.

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

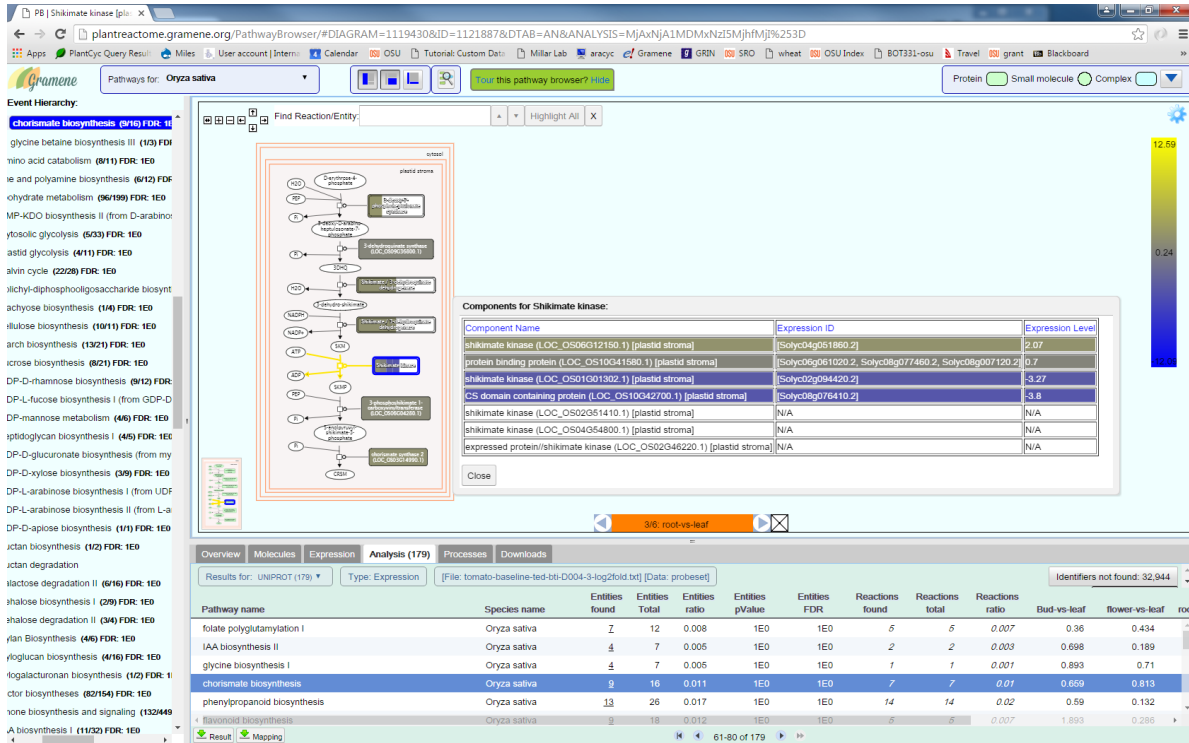
We currently provide external links for Arabidopsis, rice and Maize only. We are working on tomato and other species.

- 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



- 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.
- 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.

- 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.



- 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