

User's Guide for Plant Reactome Database (Build # 56b, Feb 2018)

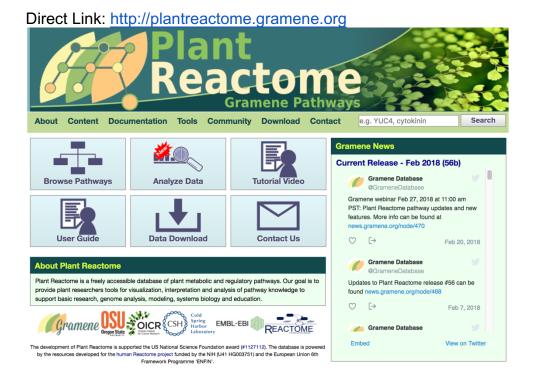
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What is Plant Reactome: The **Plant Reactome** is a free, open-source, curated and peer reviewed pathway database. Our goal is to provide intuitive bioinformatics tools for the visualization, interpretation and analysis of pathway knowledge to support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

How to access Plant Reactome?

Access is free of cost and you can click on following links to get to the plant pathways

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



What can Plant Reactome do for me?

You can find various **video tutorial in Gramene Database YouTube** channel (https://www.youtube.com/channel/UCMtmq20XMccsNUaACuqQJ-w). Specific webinars on Plant Reactome are:

Release 56b Feb 2018: https://www.youtube.com/watch?v=LsioX4x5ohk&t=1032s

Expression data analysis: https://www.youtube.com/watch?v=fyAUOBydgwI&t=58s

Plant Reactome basic: https://www.youtube.com/watch?v=d_gVlog56EQ

Plant Reactome: https://www.youtube.com/watch?v=4CkVU2Q-THs&t=50s



How many plant species are covered in Plant Reactome database: Plant Pathways are available for 75 species. We use rice as a reference species for curation and then use gene-orthology relationships to project pathways on a number of species ranging from unicellular photoautotrophs to higher plants.

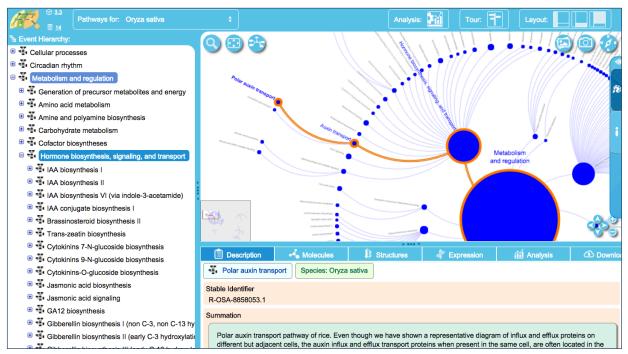
As of Release # 56b, the Plant Reactome contains gene homology-based pathway projections for 74 plant species. We regularly update our database for contents and tools. To see current species and associated statistics see http://plantreactome.gramene.org/pages/content/release-summary/

A brief summary of release 56b updates						
New and updated species projections						
	8 New species in Plant Reactome		Reference species for curation:			
	Chondrus crispus	Irish moss	Rice (0. s			
	Corchorus capsularis	white jute				
	Cucumis sativus	garden cucumber	 Orthology projections for 74 species 		s for 74	
	Dioscorea rotundata	white yam				
	Galdieria sulphuraria	a red algae		Reference	Orthology	
	Helianthus annuus	sunflower		curated	Projections	
	Lupinus angustifolius	blue lupin	Total			
	Nicotiana attenuata	coyote tobacco	Pathways	264	15,494	
	3 revised species (converted from		Reactions	1168	38,670	
	Inparanoid to Compara orthology)		Genes	1384	82,069	
	Gossypium raimondii	wild South American cotton				
	Manihot esculenta	cassava				
	Phaseolus vulgaris	common bean				

How can I see list of pathways and select a specific pathway (Browse pathway)? You can click on the "Browse Pathways" link on the PR homepage and get into Pathway Browser that lists pathways, shows a pathway diagram, and associate data on various pathway entities. Here first you will get the top-level view of pathways using Fireworks display which allows you to further select a pathway by functional category. Below are the examples of Pathway Browser views.



New "Fireworks" pathway hierarchy display

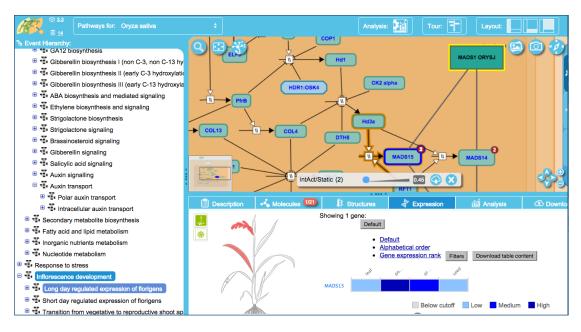


Explore pathways by functional category





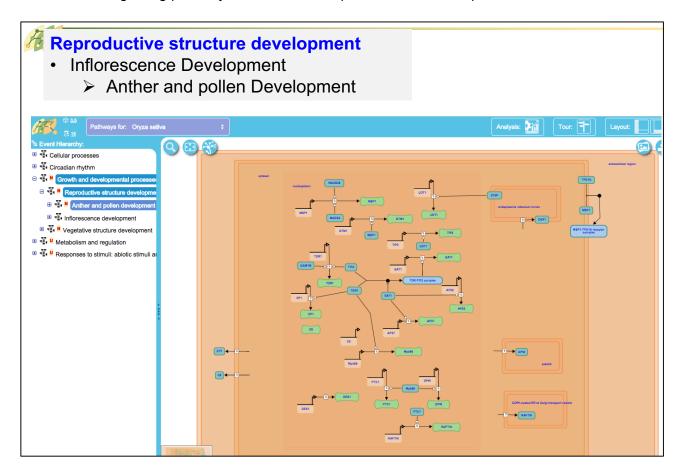
Integrates with ATLAS, Ensembl, and links to other collaborators via search (i.e. MaizeGDB, TAIR, Congenie)





What type of pathways are present in the Plant Reactome?

We have covered ~80% of metabolic pathway space. Now our focus is on curation of plant growth and developmental pathways, genetic regulatory pathways and how plants respond to various stimuli (i.e. biotic and abiotic stress). In previous example, you have seen metabolic and hormone signaling pathway. Here is an example of Anther development.

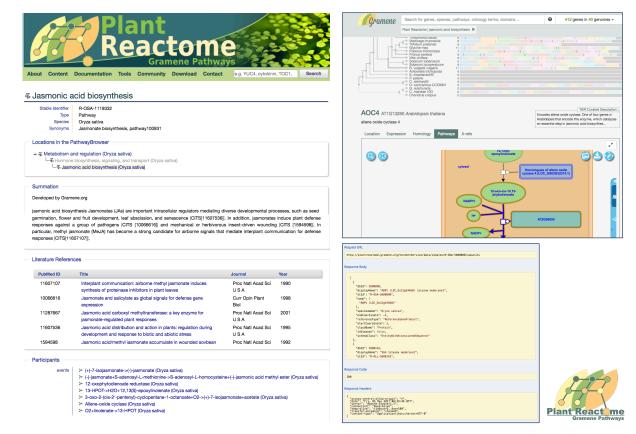


Can I identify pathways associated with gene or protein of my interest?

Yes. you can search gene or your interest in Gramene or Plant Reactome home page. Here is the summary of how you can search in the Gramene and Plant Reactome.



Extensive search and data integration features



Search for a gene or protein in Plant Reactome and find associated pathways:

Here is a specific example Use case study using Tomato COI1 gene:

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.

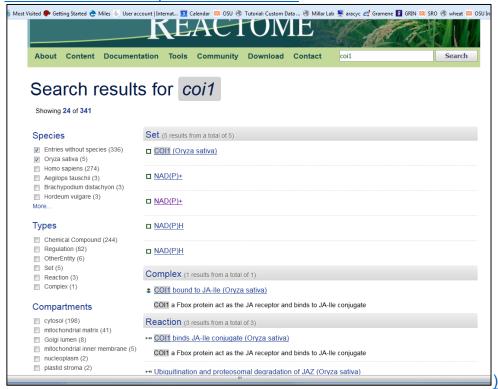
Example: 'CORONATINE-INSENSITIVE1' (or COI1), a gene required for the maternal control of seed maturation, jasmonate-signaled defense response and glandular trichome development (reference: Li et al (2004): Plant Cell, **16**:126, PMID:).





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



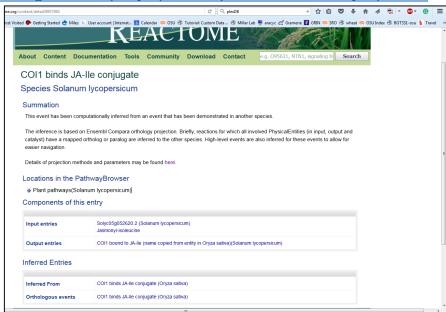


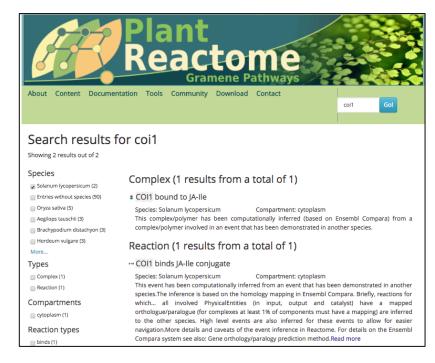
By default, the database searches are on 'Oryza sativa'. However, from the Species section, uncheck the O. sativa and entries without species and click on the 'more' option to find tomato (Solanum lycopersicum). After the selection, you will see

http://plantreactome.gramene.org/content/query?q=coi1&species=Solanum+lycopersicu

m&cluster=true

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)'.



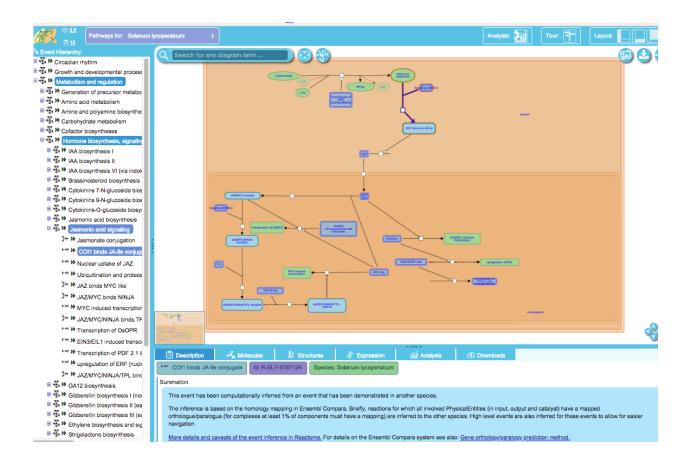


biosynthesis and signaling.

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 Pathways (Solanum lycopersicum). The hierarchy chart opens up telling about the pathways this reaction is associated with. In this case, the Jasmonic acid



http://plantreactome.gramene.org/PathwayBrowser/#/R-SLY-6787011&SEL=R-SLY-6787124&PATH=R-SLY-2744345,R-SLY-2744341



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

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 that tomato gene Solyc05g052620.2 was mapped to COI1 function in this pathway.

How can I navigate to projected pathways in another plant species (or my species of interest)?

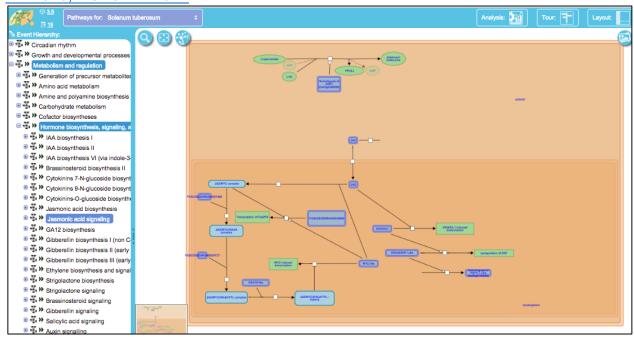
Can I find a similar event in Potato? And find COI1 gene homologs.



A. Fill in your answer.....

On the Pathway Browse page, select 'Solanum tuberosum' from the species drop down list. As soon as you select the potato species, the browser view will refresh.

http://plantreactome.gramene.org/PathwayBrowser/#/R-STU-6787011&PATH=R-STU-2744345,R-STU-2744341



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.

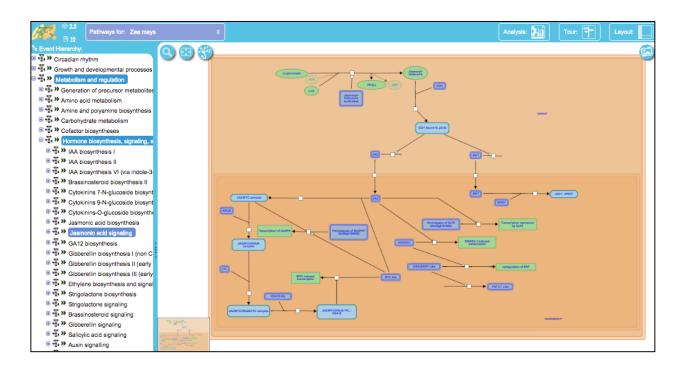
Projections in other species:

Let's try another species 'Zea mays', we see the reaction being projected in maize as well.



Select Zea mays

http://plantreactome.gramene.org/PathwayBrowser/#/R-ZMA-6787011&PATH=R-ZMA-2744345,R-ZMA-2744341



Can I compare a pathway between reference species rice and anyone of the 74plant species for which pathway projection are available?

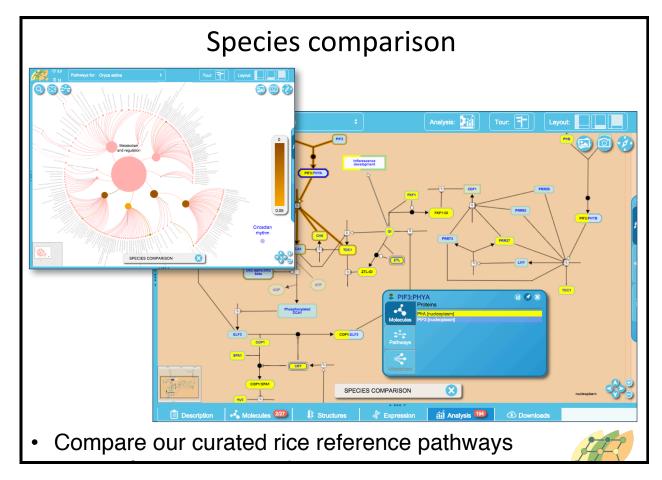
Step 1: Open browser and navigate to a pathway page e.g. Jasmonic acid signaling pathways in reference species rice (*Oryza Sativa*).

Step2: Click on the Analsis (icon on the top; above the pathway diagram). A new window with Analysis Tools menu

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

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 orthologs 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. <u>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.</u>

Users can further download results to view gene-to-gene mappings between the two species and detailed pathway and reaction info.

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

Users can click on the "molecules" icon below the pathway diagram window and then click on the Proteins to list the name of the protein coding genes. By clicking on an individual gene, will take them to Gramene gene and genome browser window, where they can access all the functionalities of genome browser and the genomic diversity

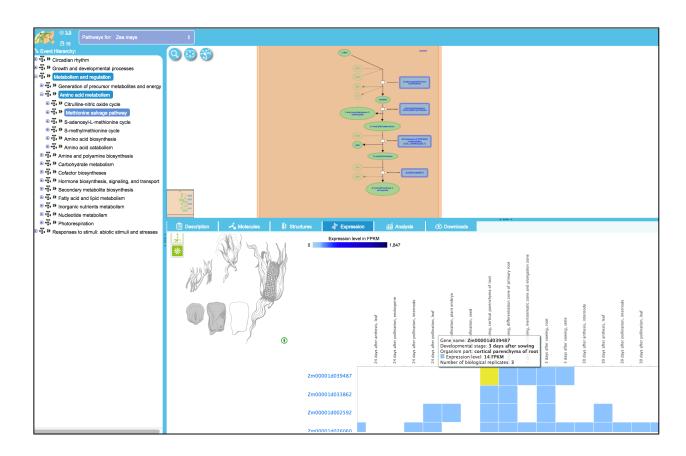


data from tomato an example is shown below: (Please refer to the genomic exercise to find out how you can find information related to gene, genome, genomic data).

If, user click on chemical compound and then select a particular compound the external page for compound ChEBI (example:

http://www.ebi.ac.uk/chebi/searchld.do?chebild=CHEBI:37569) will open up.

Similarly, clicking on the Expression tab (located below the pathway diagram) will show a baseline transcription profile of the genes associated with a given pathway. Here we show an example for maize: showing basal expression profile of genes.



Currently, expression data feature is not available for all species. This data is fetched remotely from EMBL-EBI's Plant Gene Expression Atlas.

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:

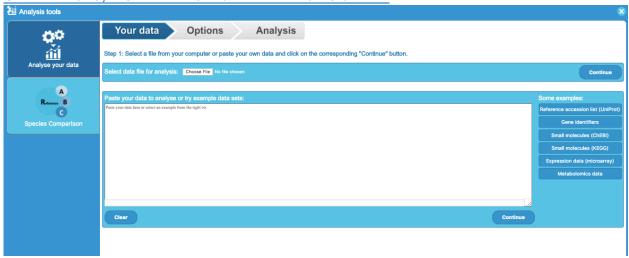
10.1038/nature11119). The tomato genome sequence provides insights into fleshy fruit evolution.

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*

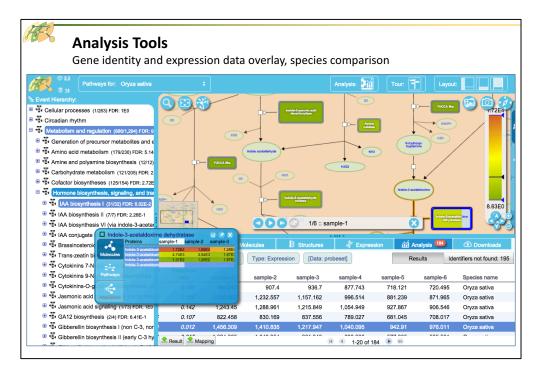
- 1. Go to the pathway browser page of your favorite species, for example, tomato
- Click on Tools-→ Choose a data file to upload (under Analysis Tools) window or enter your data into the text box (as shown in the following figure) http://plantreactome.gramene.org/PathwayBrowser/#/R-SLY-6787011&PATH=R-SLY-2744345.R-SLY-2744341&DTAB=AN&TOOL=AT



3. After uploading/ pasting the data, click on analyze to map gene expression data on Tomato.



You may get a similar view.



You can see coloring of the proteins according to the expression values. And if you click on a given enzyme or protein a pop-up window would show the difference in the homologous genes mapped to the same reaction. Considering the presence of gene duplications in plant genomes, this features helps to identify candidate genes responsible for a certain activity in a given tissue or in response to a stress condition.

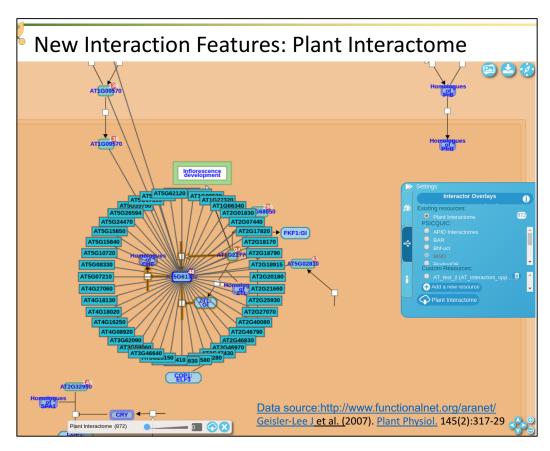
- 4. Users can browse the left-hand side panel to select an individual pathway, such as Chorismate biosynthesis pathway to see the expression of genes mapped to a pathway.
- 5. The expression data mapped to the various pathways in plant Reactome can also be downloaded.

Interaction overlays: New functionality to extend pathway gene-networks

We have built this new functionality that can bring new interaction data on the fly and can be overlaid on the pathways. Additional genes projected on pathways can help you to formulate a better hypothesis for your research.

Here is an example of ATH interaction data, brought from Aranet gene-gene interaction data. We look forward to provide similar data for other plant species, and would like to seek your help from the community to identify high-quality gene-gene interaction data for their species of interest.





If we don't currently have data for your species available in the Plant Reactome, you can always upload your own data and conduct analysis in the context of Plant Reactome.

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. However, you can download the pathway diagram, list of genes/ proteins / pathways and the results of your analysis by clicking on the 'Download' icon located in the bottom right-hand side panel.

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

Can I learn curation and seek one-to-one help from Gramene curators?

Yes, we have regular webinars, and onsite booth in PAG and ASPB conference. You can also participate in our upcoming pathway Jamboree.



