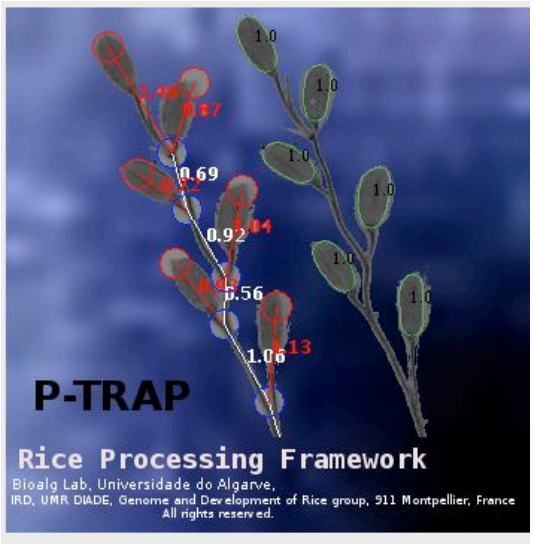
P-TRAP: Panicle Traits Phenotyping Tool

User Manual - Version October 2015

Faroq AL-Tam[1] : faroq.al.tam@gmail.com, Helene Adam[2]: adam.helene@gmail.com, António dos Anjos[4,5]: antoniodosanjos@gmail.com, Mathias Lorieux[3]: mathias.lorieux@ird.fr,Pierre Larmande [2]:Pierre.Larmande@ird.fr, Alain Ghesquière[2]: alain.ghesquiere@ird.fr, Stephane Jouannic[2]: stephane.jouannic@ird.fr Hamid Reza Shahbazkia[1]: shahbazkia@gmail.com

1- DEEI-FCT Universidade do Algarve, 8005-139 Faro, Portugal
2- IRD, UMR DIADE, Genome and Development of Rice group, 911 avenue agroplois, 34000 Montpellier, France
3- CIAT, Agrobiodiversity and Biotechnology Project, Cali, Colombia
4- DCT, ISMAT - Instituto Superior Manuel Teixeira Gomes 8500-508 Portimão, Portugal
5- CCMAR-CIMAR Laboratório Associado Universidade do Algarve 8005-139 Faro, Portugal



contact: stephane.jouannic@ird.fr / helene.adam@ird.fr

Application website:
http://bioinfo.mpl.ird.fr/index.php?option=com_content\&view=article\&id=6

Contents

Contents

List of Figures

Chapter 1 : Installation and Main Components

1 Introduction 6

1.1 System Requirements.....6

1.2 Installation 6

1.2.1 Linux..... 6

1.2.2 Windows..... 6

2 Files..... 7

2.1 Sources Images..... 7

2.2 Structure and Grains output Files..... 8

Chapter 2 :Working with P-TRAP

1 Introduction..... 10

2 Main Window..... 0

2.1 Commands 10

2.2 Project Manager..... 11

2.3 Workspace 11

3 Tasks..... 13

3.1 Getting Started 13

3.2 Adding Images to P-TRAP..... 14

3.3 Working with Images..... 16

3.4 View the Source Images 16

3.5 Crop an Image 17

3.6 Scaling Images 17

3.7 Working with the Panicle Structure..... 18

3.7.1 The Structure of a Single Image..... 18

3.7.2 The Structure of Multiple Images 19

3.7.3 View and Edit the Structure 19

3.8 Working with the Grains 21

3.8.1 Detecting the Grains in a Single Image 21

3.8.2 Detecting the Grains in Multiple Images 22

3.8.3 Color-Segmentaion for grains detection 22

3.8.4 View/Editthe Grains Files..... 22

3.9 Collecting the Reports 23

3.10 Options 23

3.10.1 Defining the Scale 24

3.10.2 Image's Background..... 25

Troubleshooting..... 25

Appendix XML Files

List of figures/Table

Chapter 1 : Installation and Main Components

1.1 The Linux installation window of P-TRAP.....6

1.2 The Windows installation window of P-TRAP.....7

1.3 The files that P-TRAP uses/produces..... 7

1.4 Example of image used for panicle structure, spikelet/grain counting 8

Table 1.1. Definition of panicle and grain traits in CSVoutputfiles..... 9

Chapter 2 : Working with P-TRAP

2.1 The main window of P-TRAP..... 10

2.2 The tool bar of the main window 11

2.3 P-TRAP project folders 11

2.4 The images editor 12

2.5 The panicle structure editor 12

2.6 The grains editor..... 13

2.7 The grains editor can be also used for editing separated grains 13

2.8 The New Project dialog: Select the project type 13

2.9 The New Projec tdialog :Nameandlocationoftheproject 13

2.10 A new project named“RicePanicleSet IRD2010”iscreated 13

2.11 The Opendialog:Add images to the project 14

2.12 New images are imported to the project..... 15

2.13 View/editimage in the Image Editor.....16

2.14 Zoom in/out in the scene in the Image Editor 16

2.15 Image editor menu..... 17

2.16 Scale images in a selected project 17

2.17 Structure detection notifications 18

2.18 A structure file generated after the image processed 18

2.19 Circles context 19

2.20 Defining the start and end generating circles 20

2.21 An error hint generated by the application to allow the user locate the error easily 21

2.22 A grains file generated from process in a panicle image..... 21

2.23 The anchors of the grains 22

2.24 Grains number correction 23

2.25 The panicle options dialog.....24

2.26 Defining the scale in image 25

Chapter1: Installation and Main Components

1. Introduction

P-TRAP is an application for analyzing plant panicle images. It is a cross-platform application developed in Java and designed on top of Netbeans Platform 7.1.
It allows the user to process the images in a project-based approach. The images to be processed are added to a project and when analyzed, the intermediary results are saved in separate files.
The final results can be collected in master and details files. Additionally, The panicle structure and the grains are stored in XML files which can be exported to be used by other applications.
Besides this manual, several video tutorials can be found in the application website.

1.1 System Requirements

1. Platform-independent
2. Core 2 Due or higher
3. 2 GB or more

1.2 Installation

1.2.1 Linux

Open the terminal, go to the folder where the grain-linux.sh is located and type: sh grain-linux.sh
The installer window will then appear, Figure 1.1. Once you press « Next » button, the installer will ask you where to put the application and its shortcuts.

Figure 1.1 : The linux installation window of P-TRAP

1.2.2 Windows

In Windows operating system, locate the file grain-windows.exe and open it. The installer, Figure 1.2, will open, click « Next » button and follow the on-screen simple instructions.

Figure 1.2 : The Windows installation window of P-TRAP

2. Files

In this section, the files that the application works with and generates are described. These files are described and shown in Figure 1.3.

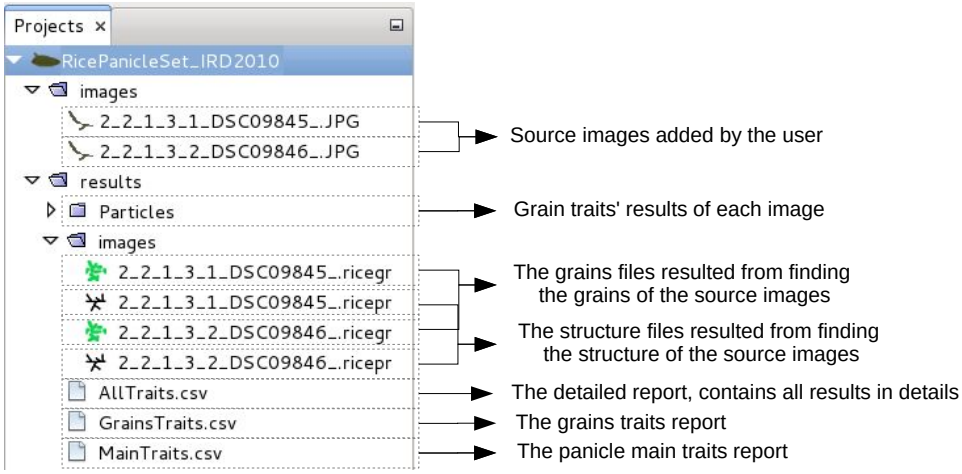


Figure 1.3 : The files that P-TRAP uses/produces

2.1 Sources Images

The application works on most of the well-known image formates. It supports both the colored and grayscale images. For better performance, it is better to scale the images as we will see in Section 3.6.
The input image is a spread panicle to separate all branches. According to the output csv file, the image has to be horizontal with the base of the panicle place on the right (orientation from right to left). Keep at least 1cm around the panicle to have a complete detection.

Figure 1.4 : Example of image used for panicle structure, spikelet/grain counting.
According to the csv output file, image has to be horizontal and panicle has to be oriented from the right to the left}

2.2 Structure and Grains output Files

The data collected from the processes images are exported in two different formats : XML and CSV.
The structure and the grains of the panicle are stored in XML files. Each analysis run produces two files : .ricepr and .ricegr for the structure and the grain respectively. These files can be used by other applications. If you are interested to use them in your application please see Appendix A(XML Files). In fact, the P-TRAP's editors described earlier uses these files to visualize the results and allow the user to visually post-process them.
These files are shown in Figure 1.3.
CSV files are also generated to allow direct visualization of the results. The results of the quantification of the panicle and grains are stored in files with two different levels of details.

These CSV files are :

- MainTraits.csv : contain the main general data about panicle structure.
- GrainsTraits.csv : contains the average values of all the data on the grain's traits. In addition to GrainsTraits.csv, each image has a result file describing each grain trait individually in the Particles folder.
- AllTraits.csv : contains detailed data on the traits of each branch.

each term is defined in the table 1.1.

Table 1.1: Definition of panicle and grain traits in CSV output

All_traits File		Grains_traits File	
Panicle Structure Trait	**Definition**	**Spikelet (Grain) tra**	**Definition**
PA_length	Primary Axis length	Sp_nb	Total Spikelet number
PA_diameter	Primary Axis diameter	Sp_length	Mean of Spikelet length
Node_nb	Nodes number on Primary axis	Sp_width	Mean of Spikelet width
SA_nb	Total Secondary axis number	Sp_area	Mean of Spikelet area
SA_average	Mean of Secondary axis length	Sp_primeter	Mean of Spikelet perimeter
SA_int	Mean of secondary axes intervals length on primary axis	Sp_circularity	Mean of Spikelet circularity
TA_nb	Total Tertiaty axis number	Sp_compactness	Mean of Spikelet compactness
TA_average	Mean of tertiary axis length	Sp_ellipticity	Mean of Spikelet ellipsity
TA_int	Mean of tertiary axes intervals length on secondary axis	Sp_AR	Aspect-ratio
QA_nb	Total Quaternary axis number		
Sp_nb	Total Spikelet number		
SA	Secondary Axe position		
SA_length	Secondary axe length		
node	number of nodes in the SA		
TA_nb	Tertiary axis nb in this SA		
Sp_nb	Spikelet nb in this SA		
SA_int	Secondary axe intervals length		
TA	Tertiary Axe position		
TA_length	Tertiary axe length		
QA_nb	Quaternary Axis number in this TA		
Sp_nb	Spiklelet nb in this TA		
TA_int	Tertiary axe intervals length		
QA	Quaternary Axe position		
QA_length	Quaternary Axe length		
Sp_nb	Spikelet number in this QA		
QA_int	Quaternary axe intervals		

Main_traits File	
Panicle Structure Trait	**Definition**
PA_length	Primary Axis length
PA_diameter	Primary Axis diameter
Node_nb	Nodes number on Primary axis
SA_nb	Total Secondary axis number
SA_average	Mean of Secondary axis length
SA_int	Mean of secondary axes intervals length on primary axis
TA_nb	Total Tertiaty axis number
TA_average	Mean of tertiary axis length
TA_int	Mean of tertiary axes intervals length on secondary axis
QA_nb	Total Quaternary axis number
Sp_nb	Total Spikelet number

Chapter2: Working with P-TRAP

1. Introduction

This chapter provides a step-by-step illustration for using P-TRAP. It is divided to a set of examples. In each one a specific task is addressed and explained.
The work with P-TRAP is very simple. The idea is to create a project that contains several images with the same scale and background intensity (\emph{i.e} dark or light background). Then process the project with options suitable for its images.

2. Main Window

The main window of P-TRAP is shown in Figure 2.1. This window has three main areas as explained in this figure.

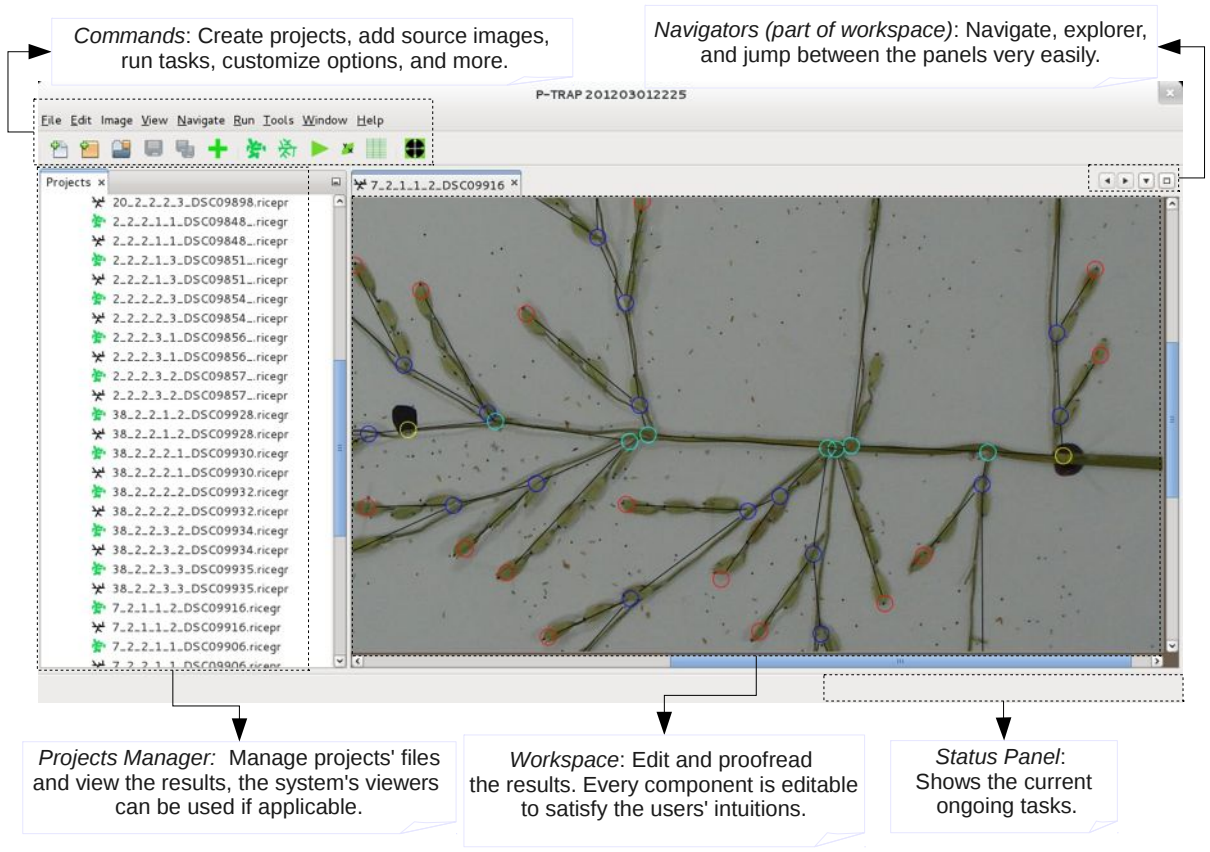


Figure 2.1 : The main window of P-TRAP.

2.1 Commands

In the *commands* area, the tool bar has several buttons with different functionalities explained in Figure 2.2.

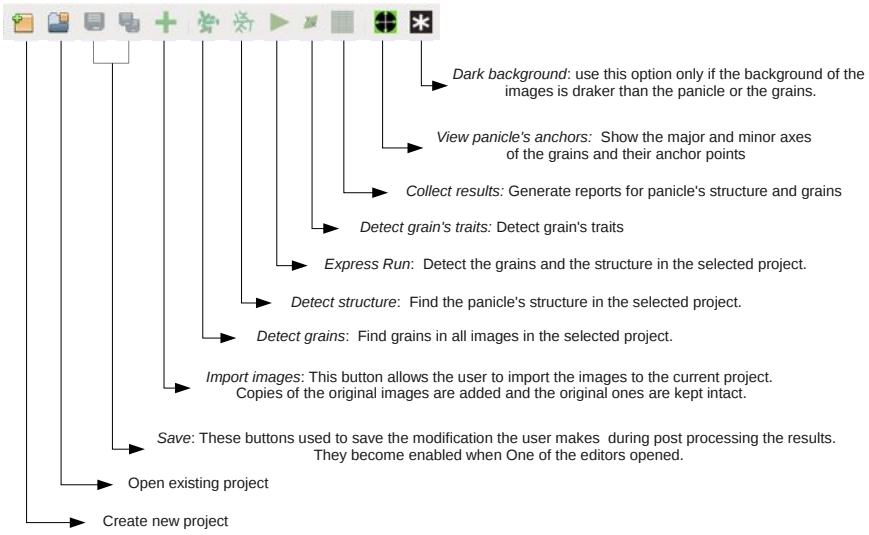
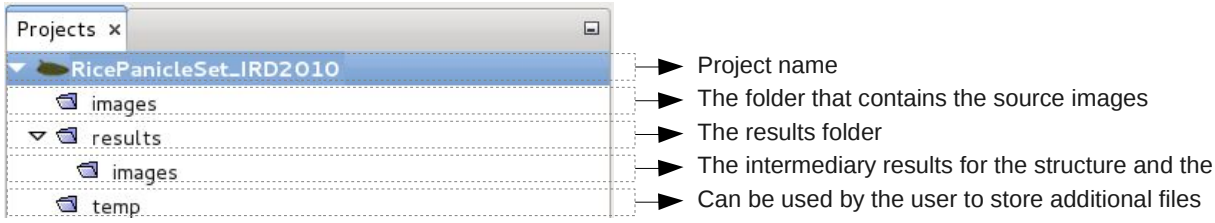


Figure 2.2: The toolbar of the main window.

2.2 Project Manager

In this area, Figure 2.3, all files and folders in the project can be dealt with very easily.
A context menu (right-clicked menu), Figure 2.3.b, can be used to manage the files.

(a)



(b)

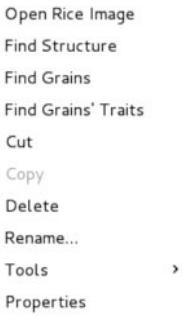


Figure 2.3 : P-Trap project folders. A) Project components; b) Projects components menu.

2.3 Workspace

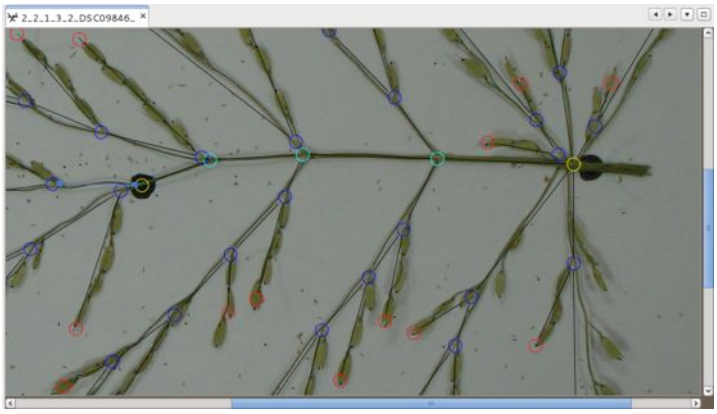
In Workspace, the user can view and edit: images, structure and grains' results. In this area, specialized editors for the images, structure and grains are available.
These editors share the same commands for zooming and moving the scene (In the editor, the scene is the entire area where the user can work).
The ImageEditor, Figure 2.4, allows the user to select the region-of-interest and crop the image.

Figure 2.4: The image editor.

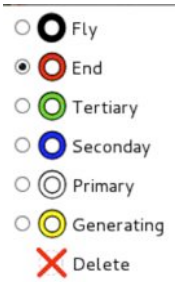
In order to crop the image, first select the area where the panicle is, then click « save » button.

By using the handles of the cropping frame the user can easily choose the exact area to be crop out.

(a)



(b)



(c)

Figure 2. 5: The panicle structure editor. a) Structure editor; b) structure editor menu; c) Structure components menu.

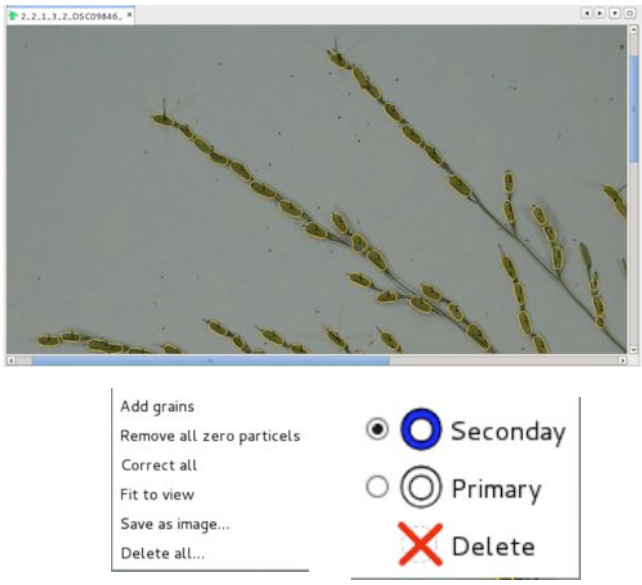


Figure 2.6: The grain editor

This editor is used for both editing the detected branches on the panicle (Fig 2.5), the grains detected on the branches of the panicle (Fig 2.6) or the grains used for detecting grains' traits (Fig 2.7).

Figure 2.7: The grains editor can also be used for editing separated grains.

3.Tasks

3.1 Getting Started

In this section, the creation of a new project is explained. To create a project do the following:-

- Open the application
- Click on the New Project button
- In the New Projectwizard, Figure 2.8, choose « Rice Processing Project » and click « Next » button.
- Specify a name and where the project will be stored, Figure 2.9.
    - \begin{figure}

- \centering
- **\includegraphics**[scale=0.15]{figures/projectName.eps}
- \caption{**\label{fig:projectName}** The \emph{New Project} dialog: Name and location of the project}
- \end{figure}

- Click « Finish ».

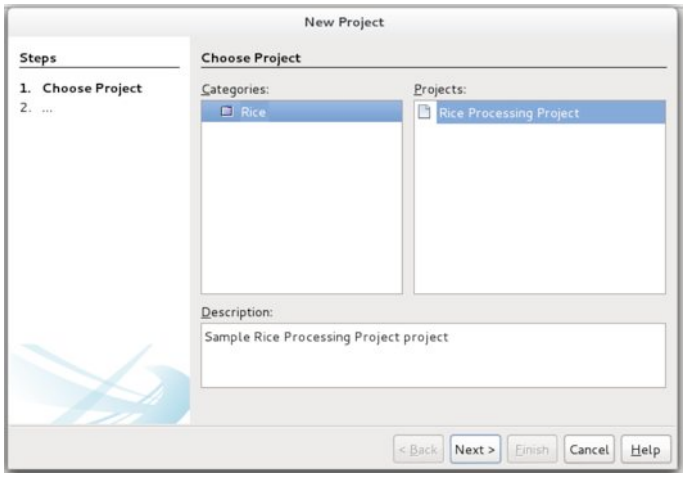


Figure 2. 8: The new project dialog ; Select the project type

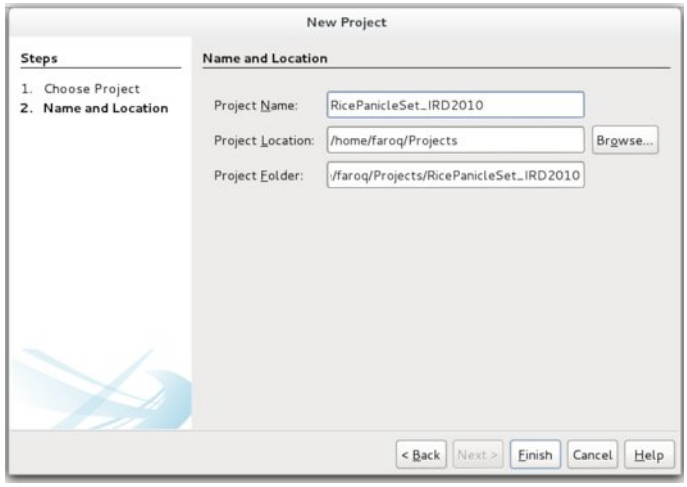


Figure 2.9: The new project dialog : Name and location of the project.

A new project will be then created as shown in Figure 2.10.

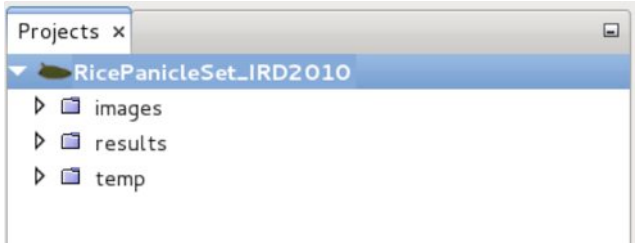


Figure 2.10: A new project named »Rice PanicleSet_IRD2010 » is created.

3.2. Adding Images to P-TRAP

In this step one or more images will be imported to the project. To do that, follow these steps:-

- Click on the Import Images icons :
- In the Open dialog, Figure 2.11, locate your images files and click OK.

You can choose single or multiple files to add to the project. Hold ctrl or shift keys during clicking on the files to select/deselect multiple file.
To select all files, press ctrl+A key combination.

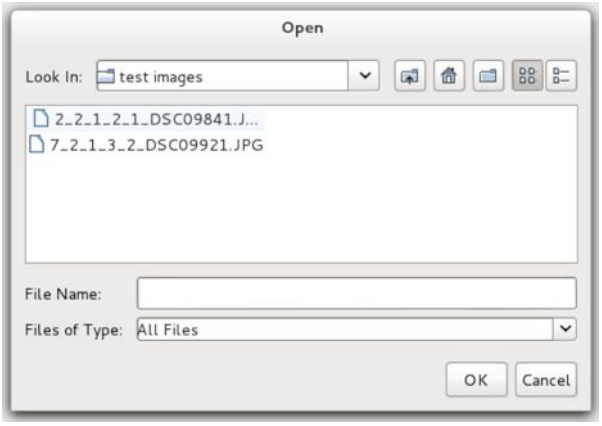


Figure 2.11: The open dialog ; Add images to the project.

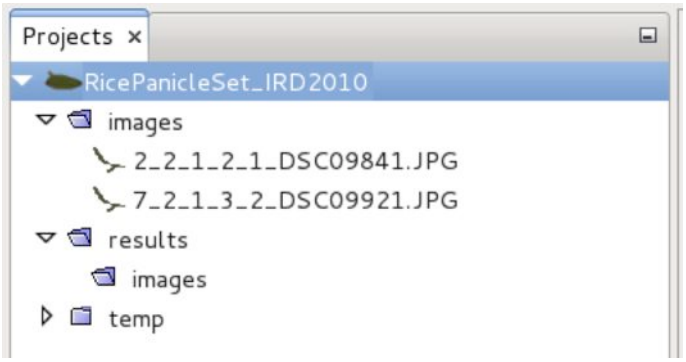


Figure 2.12: New images are imported to the project.

- Click OK, the image will be added to the « images » folder in the project, Figure 2.12.
- Click finish.

It is important to note, at this point P-TRAP does not uses the original images. Instead, it imports a copy of the original ones. So the original images are kept intact.

3.3 Working with Images

This step has some optional tasks if your images do not have huge size (we consider more than 1024×1024) as a big image) and do not have labels or additional hand-made marks.

3.4 View the Source Images

To view an image you can do one of the following:-

- Double click on the image file in the project, or
- Right click on the image, a context pop-up menu appears, choose Open Rice Image, Figure 2.3b.
- The Image Editor will then open the image and let you tweak it, Figure 2.13.

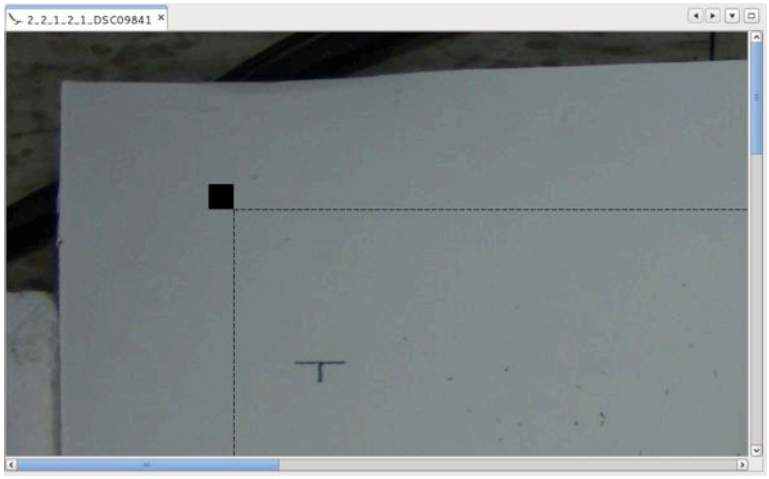


Figure 2.13: View/edit image in the Image Editor.

- Hold the ctrl+mouse wheel to zoom in/out the scene, Figure 2.14.

Please note that, this key/mouse combination can be used in all editors in P-TRAP to perform the zoom in/out task.

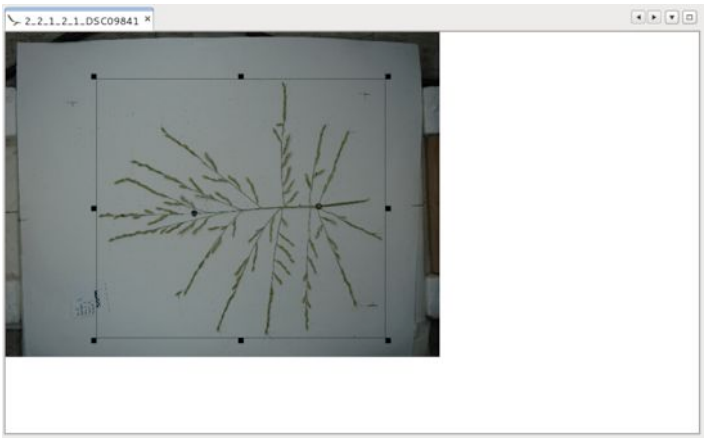


Figure 2.14 : Zoom in/out in the scene in the Image editor.

Besides, this editor has a popup menu to select the color of the grain and background (Figure 2.15). This is be used in grain detection task later on this chapter.

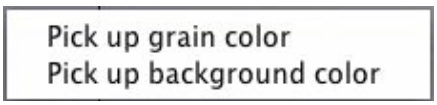


Figure 2.15 : Image editor menu.

3.5 Crop an Image

- Select the cropping area on the image by using the movable and resizable frame, see Figure 2.14.
- Click

3.6 Scaling Images

This step allows you to scale your images if they have huge or very small sizes, otherwise it is not necessary.
To scale images do the following:

- Select the project name.
- Go to the Tools menu and select « Scale images », Figure 2.16a.
- In the scaling dialog, choose the scale factor depending on the scale. you want to work with and click OK, Figure 2.16b

(a) **(b)**

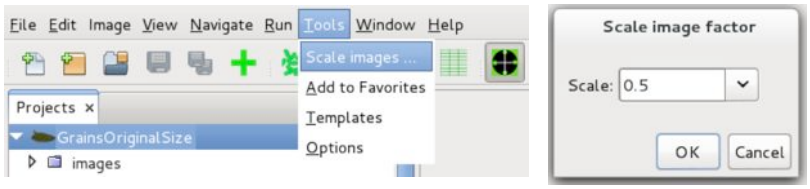


Figure 2.16: Scale images in a selected project ; a) Scale images command ;
b) Scale images factor dialog.

Usually, 1024*1024 images are enough for good processing. However, huge-sized images have to be scaled down for better processing, especially for the grain detection task. Therefore, it is strongly recommended to scaledown the images if the grain detection detects only partial parts of the grains.

3.7 Working with the Panicle Structure

This section describes how to find the structure both for a single image and for all images in the project.

3.7.1 The Structure of a Single Image

To process a single image in order to find the structure, do the following:

- Select the image file and right click to open the pop up menu, Figure 2.3.
- Choose « Find Structure »

During processing the panicle image, a progress bar in the status panel of the main window shows the current situation, Figure 2.17a.
When the processing finished the system will notify you by a message as shown in Figure 2.17b.

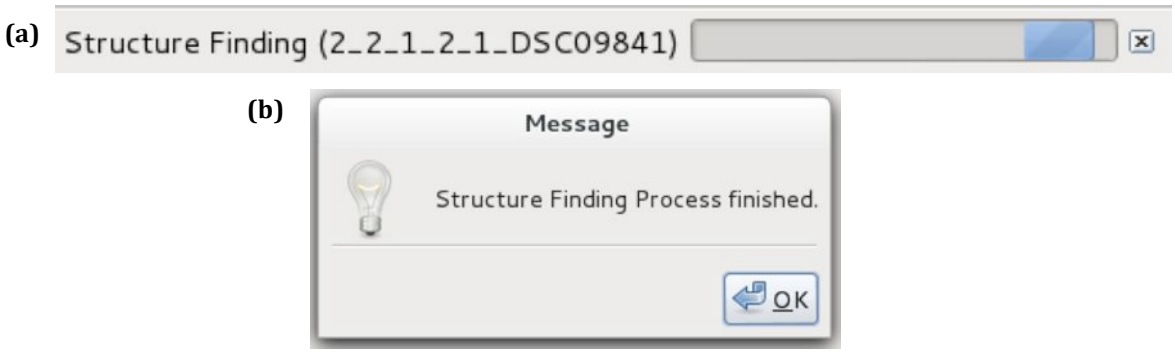


Figure 2.17: Structure detection notifications; a. Processing process; b. Structure processing notification message.

When the processing finished, a structure file will be added to the result/images folder, Figure 18.

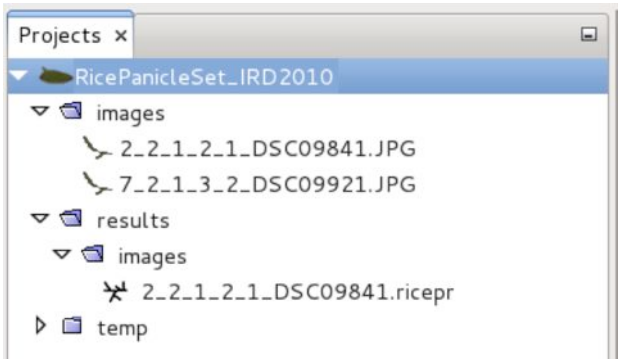


Figure 2.18: A structure file generated after the image processed}

3.7.2 The Structure of Multiple Images

- Select the project
- Click on button.

3.7.3 View and Edit the Structure

This is a very important task because it allows the user to manipulate the structure. To view the structure of a given image, do the following:

- Double click on the structure file in the project, or
- Right click on the image, a context pop-up menu appears, choose « View/edit results file » menu item.

Use the key-mouse ctrl+ mouse wheel combination to zoom in/out the scene. To move the entire scene hold the mouse wheel down and move the mouse.

To edit the structure, Table 2.1 shows the required key/mouse combinations:

Table 2.1: Structure editing key/mouse combinations.

Functionality	Command
Add a circle	Double click
Delete a circle	Right click then choose « Delete », Figure 19
Connect two circles	Hold ctrl key down then while pointing on one circle drag the mouse
Remove a link	Click on the link, then move one of its ends to an empty place
To change the type of a circle	Right click then choose the type you want, see Figure 19

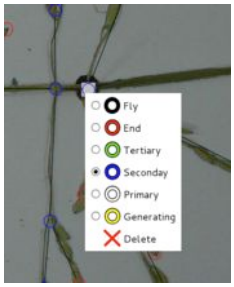
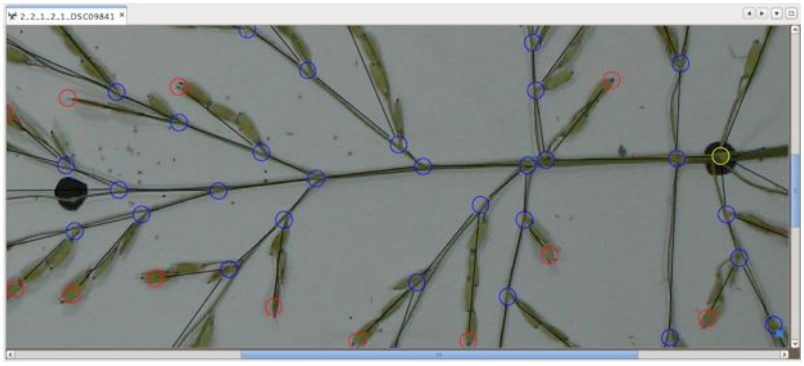
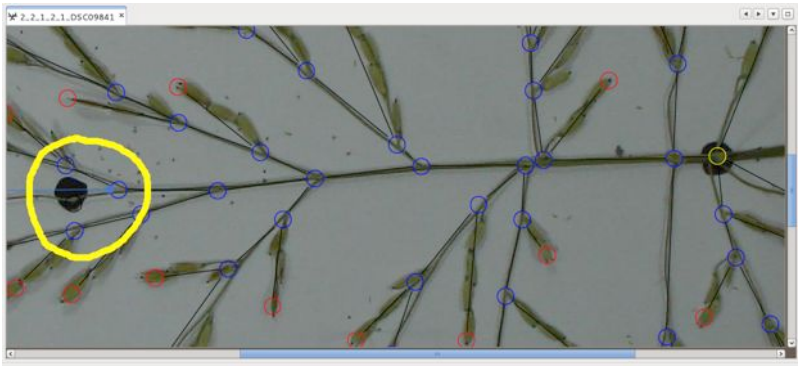


Figure 19 : Circles context menu

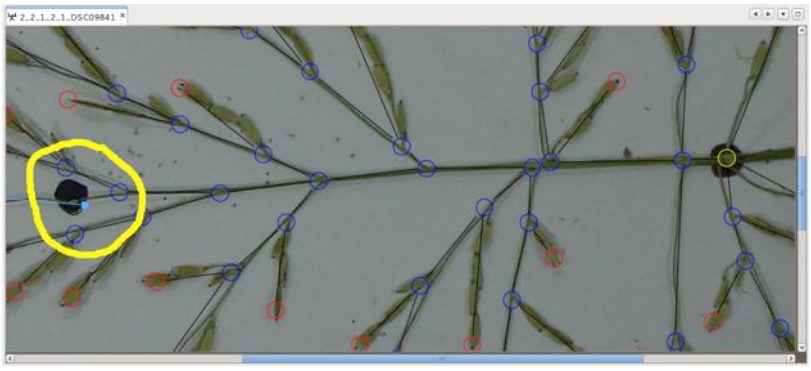
Due to the variation from one panicle to another, **the user has to define the start and end generating points (yellow-colored circles)**. This is described in details in Figure 20:



a. Select the start generating circle, right click, and then choose « Generating » from the menu



b. If the end generating point is not detected, double click to create a flying circle (i.e black).



c. Connect the new circle\end

d. Right click on this new « connected » circle and choose « generating « from the menu

Figure 20: Defining the start and end generating circles.

During post processing the results, the application will show hint if some circles need to be connected, Figure 21.

Figure 21: An error hint generated by the application to allow the user locate the error easily.

Once you finished the editing and specifying the start and end generating circles, click to save the changes you have made.

3.8 Working with the Grains

There are two methods to detect the grains. The first one deals with the clustered grains and is able to detect the grains on the branches. The other method deals with separated grains.
This section deals with both cases. In the beginning we explain how to find the grains of a single image, then the post-processing is explained.

3.8.1 Detecting the Grains in a Single Image

To process a single image in order to find the grains, do the following:

- Select the image file and right click to open the pop up menu, Chapter1, Figure 3b).
- Choose « Find Grains » if the image has clustered grains, otherwise choose « Find Grains' Traits »

Once the application finished finding the grains, it will notify the user by a message. As a result, a grain file will be generated as shown in Figure 22.

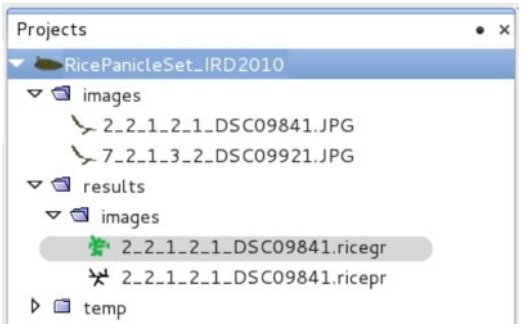


Figure 22 : A grains file generated from processing a panicle image

3.8.2 Detecting the Grains in Multiple Images

- Select the project.
- Click button, if the grains are clustered. Otherwise click button.

A progress bar will appear in the right bottom of the main window to show the percentage of images being processed. Once it finished, it will show a notification message.

3.8.3 Color-Segmentaion for grains detection

In some cases, the main automatic segmentation method may have problems finding the accurate bounds of the grains. For this propose a manual-guided method is available. In this method, the user has to specify the grain and background colors.

To define these colors:

- Open the image in the image editor.
- Right-click to open a pop up menu as shown in Figure 15..
- Select the colors.

To run in color-segmentation mode, toggle ON the color segmentation,

3.8.4 View/Edit the Grains Files

To view a grain file do one of the following:

- Double click on the grain file in the project, or
- Right click on the image, a context pop-up menu appears, choose « View/edit grains file}.

To view the grains major and minor axes, Figure 23, toggle the ON.

Figure 23: The anchors of the grains.

Editing the grains file includes adding, removing, and correcting the number of grains. To add a grain, right click in the place you want to add a grain and then choose « add grain ». To remove a grain or a grains' cluster, right click on the grain and then choose « remove grains ».
For modifying the number of grains, double click on the grain then write the correct number of grains, Figure 24.

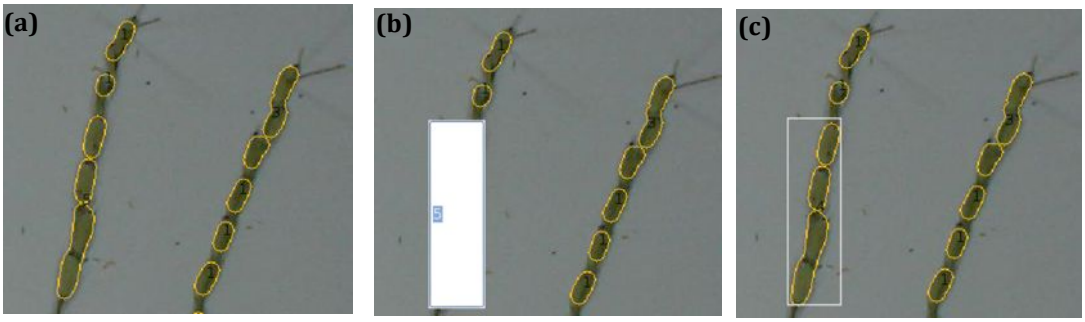


Figure 24 : Grains number correction. a. Incorrect number of grains ; b. Edit the number of grains ; c. grains number correction.

Once you finish post-processing the grains, click the save button to save the changes you have made.

3.9 Collecting the Reports

As mentioned before, P-TRAP generates three reports in Comma Separated Values (CSV) format. Once the processing and post-processing tasks are performed, the final reports can be generated by:

- Select the project
- Click button.

When the application finished collecting the results of all images, it will notify the user by a message. The reports files are shown in Figure 3.

3.10 Options

P-TRAP offers a set of options for the graphical interface and the core process as well. This sections describes the processing options.
The panicle options can be found in: Tools/Options menu, then choose Panicle Options, Figure 25. These options are explained in Table 2.2.

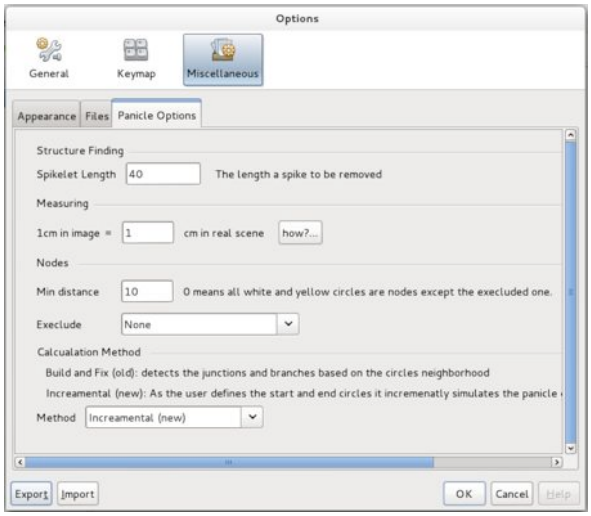


Figure 25 : The panicle option dialog

Table 2.2: P-Trap panicle options.

Option	Description
Structure Finding	
Spikelet Length	Controls the minimum length of the branch, any branch that has length less than this value will be mark as a noise and removed.
1cm	This option defines the ratio between the cm in the image and in th real panicle.
Nodes	
Min distance	Controls the minimum distance between two nodes in the main axis in the panicle. If the distance between two nodes is less than this value they are counted as a single node.
Exclude	Which nodes has to be excluded from counting?
Calculation Method	
Method	Which method that applications has to use in order to detect the panicle structure? Usually the incremental method works better.

3.10.1 Defining the Scale

The scale label is widely used in many biological work. P-TRAP allows the user to define the measuring scale in the image.
The following steps explain how to define the measuring scale :

- Open the image that contains the scale by double-clicking on the image.
- Drag from the one end of the scale, Figure 26a, and hold until you reach the other end then right click, Figure 26b.
- The scale dialog appear, Figure 26c define how many unite in the scale and click « OK ».
-

The application will then use the ratio between the real panicle size and the panicle size in the image to make accurate calculations.

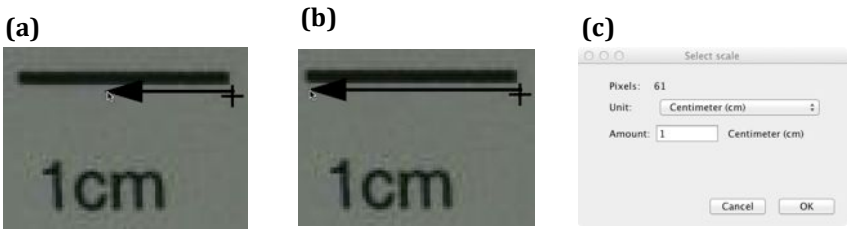


Figure 26 : Defining the scale in image. a. Drage on one end of the scale ; b. Righ-click once the other end is reached ; c. Define how many unites in the scale.

3.10.2 Image's Background

In some cases, the images backgrounds are darker than the objects. Therefore, P-TRAP offers an option such that the user defines the type of the image's background.
The default option is white, you can change it to dark by toggling the « Dark Background » button ON.

4. Troubleshooting

- Not very good detection.

1- Try to scale down the images if they are very large or crop the unwanted background or artifacts at the borders.

2- If the background is dark try to use the « dark background » .

- Unable to save the detected structure. Try to remove all flying circles (black colors) you can use the right-click menu to remove them all. Also make sure you have determined the start and end generating points.

- The report is empty.} Make sure you have defined the start and end generating points in the structure.
- Aberrant csv outfile} Make sure you have defined the start and end generating points in the structure.

Make sure of the orientation of the image (see chapter 1, 2.1)

Appendix A. XML Files

1. Structure XML File

The structure XML file is compactly describing the structure of the panicle as a mathematical graph composed of
vertices and edges. What is needed to use this file is any XML parser plugged in your application to
convert this file into your graph data structure, by tackling only the <graph> entity and its components.
The entire structure of the file is described as the following:

- <result signature=``STRUCTUE'' imagepath=``18_2_2_1_2_DSC09873.JPG''>

The is the main tag, which means this is a structure result file identified by the signature, ``STRUCTURE''.

- <graph>

Describes a graph of vertices and edges, this graph represents the panicle structure.

- <vertices>

contains a set of vertices (these are converted to cycles as you can see them in the application). They describ the junctions and the ends of the panicle structure.

- <vertex id=``java.awt.Point[x=994,y=1282'' x=``994'' y=``1282'' type=``End'' fixed=``false''/>

  A single vertex. Its attributes are:
  id: an identifier of the vertex,
  - x and y: the xy-coordinates of the vertices,
  - type: the type of the vertex,
  - isfixed: true if the vertex has been edited by the user.

- <edges>

  The edges that connects the graph vertices
- <edge vertex1=``java.awt.Point[x=1047,y=1053]'' vertex2=``java.awt.Point[x=1149,y=1015]''/>

  an edge that connects two vertices given their identifiers ``vertex1'' and ``vertex2''.