

Supplementary material of the manuscript published in *Vitis* **61**, 71–76 (2022):

The effect of homozygosity of locus *Rpv12* on downy mildew resistance of grapevine leaves

S. MÜLLNER and E. ZYPRIAN

Julius Kühn-Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany

Macro for Mycelial growth of reference genotypes analysis using Fiji

In case of stronger mycelial growth (reference genotypes 'Italia', 'Afus Ali' as well as 'Kunbarat') for this macro the blue colour channel was used. First, the colour channels were split and the red and green channels were discarded. The blue channel was analysed using the automatic threshold filter "IJ_IsoData dark". The macro was created with the support of M. Sc. T. PROKSCH.

```
//Create file path dialogue, to choose file path

parDir = getDirectory("Choose a Directory");

if (parDir=="")

exit("No directory available"); //get directory with images

Dialog.create("Saving Options");

//Create file list with all the files of chosen folder list = getFileList(parDir);

//Run program for all files in folder

for (i=0; i<list.length; i++)

{

//to set file name

fullName = parDir + list[i];

//if filename ends with .jpg..

if (endsWith(fullName, ".jpg"))

{

//1. Open image and mark Imagename

open(fullName);

Bildname = getTitle;

//2. Divide colors. Discard red and green      run("Split Channels");

selectWindow(Bildname + " (red)");

close();

selectWindow(Bildname + " (green)");

close();
```

//3. Spreading the contrast to fill Histogramm completely

```
selectWindow(Bildname + " (blue)");
```

```
getStatistics(area, mean, min, max);
```

```
run("Brightness/Contrast...");
```

```
setMinAndMax(20, max);
```

```
run("Apply LUT");
```

```
run("Close");
```

//4. Threshold filter

```
setAutoThreshold("IJ_IsoData dark"); // sets threshold between left (background)
```

```
and right (object) maximum      setOption("BlackBackground", true);
```

```
run("Convert to Mask");
```

//5. Remove Particles

```
selectWindow(Bildname + " (blue)");
```

```
run("Analyze Particles...", "size=10000-Infinity show=Masks clear");
```

```
selectWindow(Bildname + " (blue)");
```

```
close;
```

```
selectWindow("Mask of " + Bildname + " (blue)");
```

```
run(",Invert"); // Bild invertieren: sw <-> ws
```

```
run("Analyze Particles...", "size=10000-Infinity show=[Count Masks] summarize");
```

//6. Save images

```
selectWindow("Mask of " + Bildname + " (blue)");
```

```
saveAs("jpg", "D:/ImageJ-Results-Mycelium/" + Bildname);
```

//6. Save Results

```
selectWindow("Summary");
```

```
saveAs("Results", "D:/ImageJ-Results-Mycelium/" + Bildname + ".csv");
```

```
run("Close");
```

```
close();
```

```
close();
```

```
}
```

```
}
```

Macro for Mycelial growth of homozygous genotype analysis using Fiji

In case of low mycelial growth (Hozy01, Hozy10 and IRZ0973), for the macro a difference image was created. First, colour channels were split and the blue channel (mycelium) was offset against the red channel (background). "Default dark" was used as the automatic threshold filter. The macro was created with the support of M. Sc. T. PROKSCH.

```
//Create file path dialogue, to choose file path
parDir = getDirectory("Choose a Directory");
if (parDir=="")
exit("No directory available"); //get directory with images
Dialog.create("Saving Options");

//Create file list with all the files of chosen folder list = getFileList(parDir);

//Run program for all files in folder (i=Index for files in file list) count up
for (i=0; i<list.length; i++)
{
    //to set file name (i-th file from list)
    fullName = parDir + list[i];

    //if file name ends with jpg
    if (endsWith(fullName, ".jpg"))
    {
        //1. Open image and mark imagename
        open(fullName);
        Bildname = getTitle();

        //2. Divide colors. Discard green.
        // as the mycelia have almost no red values (completely black in the red image), they
        // can be removed via a differential image (blue-red).
        run("Split Channels");

        selectWindow(Bildname + " (green)");
        close();

        // 3. Create difference image
        imageCalculator("Difference create", Bildname + " (red)", Bildname + " (blue)");
        selectWindow(Bildname + " (red)");
        close();
        selectWindow(Bildname + " (blue)");
        close();
        selectWindow("Result of " + Bildname + " (red)");

        //4. Spread contrast to fill histogram completely
        getStatistics(area, mean, min, max);
        run("Brightness/Contrast...");
        setMinAndMax(20, max);
    }
}
```

```

run("Apply LUT");
run("Close");

//5. Threshold filter: Everything below the (auto) threshold
becomes black and everything above the threshold becomes white
setAutoThreshold("Default dark");
// the auto-threshold sets the threshold between the 2 maxima
(left maximum = background, right maximum = searched object)
setOption("BlackBackground", true);
run("Convert to Mask");

//6. Remove Particles: remove everything that is smaller
than... coherent pixels
selectWindow("Result of " + Bildname + " (red)");
run("Analyze Particles...", "size=1000-Infinity show=Masks clear");
selectWindow("Result of " + Bildname + " (red)");
close;
selectWindow("Mask of Result of " + Bildname + " (red)");
run("Invert");
run("Analyze Particles...", "size=1-Infinity show=[Count Masks] summarize");

//6. Save image
selectWindow("Mask of Result of " + Bildname + " (red)");
saveAs("jpg", "D:/ImageJ-Results-Mycelium/" + Bildname);

//7. Save Results in Excel
selectWindow("Summary");
saveAs("Results", "D:/ImageJ-Results-Mycelium/" + Bildname + ".csv");
run(",Close");
close();
close();

}
}

```