

You are given 3 samples of 4 leafs with brown spots indicating thermal stress and consequent ROS production. (C3, C3 After heat, C4, C4 after heat) You have to find the expression in arbitrary units, which feature is best for determining this (1), which channel will you use in this analysis? (2) Split the channels by Image>Color>Split Channels and choose the appropriate channel for further analysis. Now, use the freehand selection tool , and try to cover the leaf area (NO PETIOLE) save your selections into the ROI MANAGER by pressing (3) . now, proceed to Analyze>Set Measurements and tick the answer to (1). Go to ROI manager and click measure. SAVE YOUR MEASUREMENTS (4). Now, tell which value indicates more expression? a higher mean gray value or a lower mean gray value (5). Now, calculate difference in before and after temperature increase mean gray value by first subtracting 255 from your obtained value and then subtracting values of after then before. (6) . Now calculate the percentage change in C3 and C4 leaves (7).What is your conclusion?

Answers:

(1) -- Mean gray value - as it will determine the average expression

(2) -- Blue channel - As the spots were brown

(3) -- Ctrl+T

(4) -- Results should be in range of attatched files.

(5) -- Lower mean gray value, as Blue channel appears darker where there was more brown color in the original file.