

Cowpea Leaf disk collection

Stephanie Cullum

Abstract

In order to take chlorophyll fluorescence images of cowpea accessions, leaf disks need to be collected and placed randomly onto a leaf disk tray. This protocol explains how.

Citation: Stephanie Cullum Cowpea Leaf disk collection. **protocols.io**

dx.doi.org/10.17504/protocols.io.rspd6dn

Published: 02 Aug 2018

Protocol

The night before collections, cut up 3 sponges into 5mm cubes.

Step 1.

Also, if possible, set up program to randomise the leaf disk collections onto the 9 by 6 leaf plate tray (if not then just randomly place them as best as you can during the collections).

For collection, take a hole punch, leaf trays, sponge cubes soaked in water (in a ziplock bag), tin foil, a small pointed object (for leaf disk positioning) and a lab book with a pen.

Step 2.

While each accession had 6 cowpea seeds planted, a few accessions had no growth while the success of other accessions varied in plant growth and number.

Step 3.

For the best quality results and to not inhibit plant growth, only take leaf disks from plants big enough to survive the damage.

Before starting collection, place sponge cubes into the leaf disk tray (one sponge cube per leaf disk hole) in such a way that they are pushed within the tray but are all at equal height.

Step 4.

Leave the last column free as this will be used as a plate number marker

For the first accession, make note of the accession number and take one leaf disk from 4 different plants within the accession

Step 5.

if not then do repeat disks on the same plant

To take a leaf disk, slide the leaf within the hole punch in such a way that the leaf disk taken will not have a vein or pest damage

Step 6.

Press the hole punch to take a leaf disk

Step 7.

After removing the hole punch from the leaf, the leaf disk needs to be collected.

Step 8.

If the hole punch works correctly then a second press of the hole punch removes the disk. However, the hole punch can be temperamental and the leaf disk may fall or stick to the punch or get stuck within the punch. The sharp pointed object can be used in such cases to free the leaf disk

Place the leaf disk into a random place within the leaf tray with the top surface of the leaf facing upwards.

Step 9.

To place the disk so that it is flat, use the pointed object to manipulate the leaf into place. Make note of the placement of the leaf disk within the tray e.g. A6

Repeat so that there is 4 leaf disks for the whole accession

Step 10.

Move onto the next viable accession and repeat until a leaf disk tray has been filled.

Step 11.

After filling the tray, place a leaf piece into the right hand column top row to indicate that this tray is the 1st.

While keeping the tray flat, wrap it in tin foil completely to dark adapt it while collecting other disks

Step 12.

Repeat for other trays until all accessions have been collected

Step 13.