An investigation of pigments present in leaves

Introduction

In plants, chlorophyll is the main pigment that absorbs light energy during photosynthesis. Most plants have other photosynthetic pigments as well and these are not green. You will be using a technique called chromatography to separate chlorophyll and other pigments from two different leaves, A and B.

Materials

You are provided with the following:

- boiling tube rack
- two boiling tubes with bungs
- small glass measuring cylinder
- solvent
- filter paper
- glass rod
- two leaves, A and B
- cork borer
- tile on which to use cork borer
- ruler
- pencil
- drawing pins
- marker pen
- sticky tape

You may ask your teacher for any other apparatus you require.

Read these instructions carefully before you start your investigation.

Method

1. Set up two boiling tubes at the start of the investigation. Add 3 cm³ of solvent to each of the two boiling tubes. Put a bung in the top of each tube and stand them upright in a rack. Label the tubes A and B.
2. Take a piece of filter paper that fits into the boiling tube, as shown in the diagram. Rule a pencil line 2 cm from the bottom of the filter paper. This line is called the origin. Write leaf A at the top of the filter paper in pencil.
3. Cut a disc from leaf A with a cork borer. Try to avoid the veins and midrib of the leaf when you do this.
4. Place the leaf disc on the filter paper at the centre of the line marking the origin. Crush the disc into the paper with the end of a glass rod. The crushed leaf disc should leave a stain on the filter paper.
5. Pin the filter paper to the bung with a drawing pin, then put the filter paper into the tube labelled A as shown in the diagram on page 2. Make sure the end of the filter paper is in the solvent and that the solvent does not come above the origin. Put the tube carefully back into the rack and do not move it again.
6. Let the solvent run up the filter paper until it almost reaches the top of the paper. Remove the filter paper from the tube and immediately draw a pencil line to show how far the solvent moved up the paper. This line marks the solvent front.

7. Replace the bung in the tube.

8. The filter paper with its coloured spots is called a chromatogram. Let the chromatogram dry. Using a pencil, draw round each coloured spot on the chromatogram.

9. Repeat step 2 with the second piece of paper but write B at the top of the filter paper.

10. Repeat steps 3 – 8 with leaf B.

When you have completed steps 1 to 10 above, attach your dried chromatogram here with sticky tape.

Leaf A

Leaf B
ISA BIO6T/P12 Candidate Results Sheet: Stage 1

An investigation of pigments present in leaves

Centre Number ____________  Candidate Number ____________

Candidate Name ...................................................................................................................................

You can calculate the Rf value of the pigments that you have isolated using the equation

\[ Rf \text{ value} = \frac{\text{Distance moved by pigment from origin to centre of pigment spot}}{\text{Distance from origin to solvent front}} \]

Select one of the pigments from Leaf A. Label this pigment on the dried chromatogram with the letter P.

1 Calculate the Rf value of this pigment.

(1 mark)

Hand in this sheet at the end of each session.
ISA BIO6T/P12 Candidate Results Sheet: Stage 2

An investigation of pigments present in leaves

Centre Number ______________________  Candidate Number ______________________

Candidate Name.................................................................................................................

Students carried out similar investigations to you with green leaves and yellow leaves. The students stopped each chromatogram when the solvent front had run exactly the same distance. They then measured the distance moved by the pigment nearest the origin. The results are recorded in the table.

<table>
<thead>
<tr>
<th>Trial number</th>
<th>Green Leaf</th>
<th>Yellow Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>54</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>38</td>
</tr>
<tr>
<td>9</td>
<td>52</td>
<td>36</td>
</tr>
</tbody>
</table>

Use a statistical test to analyse the data in the table. You may use a calculator and the AQA Students' Statistics Sheet that has been provided.

A sheet of graph paper is supplied. You may use this if you wish.

2  State your null hypothesis.

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(1 mark)
3 (a) Give your choice of statistical test.

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(1 mark)

3 (b) Give a reason for your choice of statistical test.

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(1 mark)

4 Carry out the test and calculate the test statistic. Show your working.

(1 mark)
Interpret the test statistic in relation to your null hypothesis. Use the words *probability* and *chance* in your answer.

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..........................................................................................................................................
(2 marks)

END OF QUESTIONS
AQA Students’ Statistics Sheet (Version 3)

What sort of data will you obtain from your investigation?

Measurements
Will your investigation involve taking measurements?

Will your investigation involve looking for associations between different measurements from the same sample?

Spearman rank correlation

 Frequencies
Will your investigation involve finding the number of individuals in particular categories?

Will your investigation involve looking for differences between mean values?

Standard error and 95% confidence limits

Standard error and 95% confidence limits

Calculate standard error, $SE$, for each sample from the following formula

$$SE = \frac{SD}{\sqrt{n}}$$

where $SD$ = standard deviation
and $n$ = sample size

95% confidence limits = $2 \times SE$ above and below the mean

For use in the ISA and EMPA assessment
The $\chi^2$ test

The chi-square ($\chi^2$) test is based on calculating the value of $\chi^2$ from the equation

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

where $O$ represents the results you observe in the investigation and $E$ represents the results you expect.

Table showing the critical values of $\chi^2$ at $P = 0.05$ for different degrees of freedom

<table>
<thead>
<tr>
<th>Degrees of freedom</th>
<th>Critical value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.84</td>
</tr>
<tr>
<td>2</td>
<td>5.99</td>
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<tr>
<td>3</td>
<td>7.82</td>
</tr>
<tr>
<td>4</td>
<td>9.49</td>
</tr>
<tr>
<td>5</td>
<td>11.07</td>
</tr>
<tr>
<td>6</td>
<td>12.59</td>
</tr>
<tr>
<td>7</td>
<td>14.07</td>
</tr>
<tr>
<td>8</td>
<td>15.51</td>
</tr>
<tr>
<td>9</td>
<td>16.92</td>
</tr>
<tr>
<td>10</td>
<td>18.31</td>
</tr>
</tbody>
</table>

Spearman rank correlation test

Calculate the value of the Spearman rank correlation, $r_s$, from the equation

$$r_s = 1 - \left[ \frac{6 \times \sum D^2}{n^3-n} \right]$$

where $n$ is the number of pairs of items in the sample and $D$ is the difference between each pair of ranked measurements.

Table showing the critical values of $r_s$ at $P = 0.05$ for different numbers of paired values

<table>
<thead>
<tr>
<th>Number of pairs of measurements</th>
<th>Critical value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>0.89</td>
</tr>
<tr>
<td>7</td>
<td>0.79</td>
</tr>
<tr>
<td>8</td>
<td>0.74</td>
</tr>
<tr>
<td>9</td>
<td>0.68</td>
</tr>
<tr>
<td>10</td>
<td>0.65</td>
</tr>
<tr>
<td>12</td>
<td>0.59</td>
</tr>
<tr>
<td>14</td>
<td>0.54</td>
</tr>
<tr>
<td>16</td>
<td>0.51</td>
</tr>
<tr>
<td>18</td>
<td>0.48</td>
</tr>
</tbody>
</table>