Nuclear Genome Diversity in Somatic Cells is Accelerated by Environmental Stress

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Method to measure directly the transfer rate of DNA from chloroplast to the nucleus

Kanamycin resistance product

Spectinomycin resistance


tp-neo for stable integration

Chloroplast genome transformed with pPRV111A::neoSTLS2

Frequency:
1/16,000 in pollen;
1/273,000 in ovule;
1/5million in somatic cells.


Analysis of sector frequency in leaves, roots and cotyledons in tp-gus lines and tp-gus lines treated on 45°C for 3 hours.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Leaf</th>
<th>Control</th>
<th>Heat</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.76</td>
<td>2.48</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.84</td>
<td>3.89</td>
<td>p=NS</td>
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<tr>
<td></td>
<td></td>
<td>2.69</td>
<td>4.40</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.93</td>
<td>2.76</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>4.59</td>
<td>p=NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.72</td>
<td>4.59</td>
<td>p=NS</td>
</tr>
</tbody>
</table>

Extremes of different environmental factors, such as salt, cold and heat were used.

After treatment the plants will be stained for gus activity and the number of blue spots counted.

tp-gus for stable integration and transient expression


Heat increases the transfer of chloroplast DNA into nucleus

- GUS mRNA
- Unspliced GUS mRNA

**tp-gus**

Resistant shoots recovered

**Frequency**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Control:</td>
<td>13</td>
<td>1 in 1.4 million cells</td>
</tr>
<tr>
<td>Heat treatment:</td>
<td>45</td>
<td>1 in 400,000 cells</td>
</tr>
</tbody>
</table>

3.5 fold increase

How do organellar DNAs integrate to nuclear genome in multicellular eukaryotes?

Double-strand breaks (DSBs) repair can introduce mitochondrial DNA to nuclear genome in yeast.

Is organelle DNA integration related to DSB repair in plant?

DNA double-strand break system in nucleus

Nuclear Integration of Mitochondrial DNA in the DSB break points is only found after heat treatment

Ongoing Project

Detect the Chloroplast DNA integration introduced by DSB repair in nucleus

Heat disrupts the membranes of chloroplast

Heat stress increases the flow of DNA from cytoplasmic organelles to the nucleus.

Double strand DNA breaks repair leads to insertion of organellar DNA into the nuclear genome in plant.

Summary
Acknowledgements

Jeremy Timmis                        David Adelson
Andrew Lloyd                        Jack da Silva
Mathieu Rousseau                    Sonia Dayan
Rory O’ Connor                      Zhipeng Qu
Yuan Li                             Simlin Lim
Frank Grützner

Funding
ARC Discovery Project
China Council Scholarship

Thank you