Are the sequences of the chloroplast genomes of the daffodil cultivars Ornatus and Empress different?

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Overview

Using Oxford Nanopore MinIon technology, we expect to be able to obtain the full chloroplast DNA sequence of Ornatus and Empress daffodils. Comparing these with Jalview, a sequence alignment program, we expect to see high levels of similarity but some differences in the genomes.

Ornatus

Empress

Images thanks to Croft 16 Daffodils

Aims

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Background information

Chloroplasts are plant cell organelles that are found in green plants which convert sunlight into energy that they can use – they contain chlorophyll (where photosynthesis takes place).

Chloroplasts contain their own DNA as they are descendants of prokaryotes that were taken up into eukaryotes a long time ago. All chloroplast genomes have a length between 110,000 and 200,000 base pairs.

Chloroplast genomic research has important translational applications, such as the development of vaccines and pharmaceutics in edible crop plants, protection against biotic and abiotic stress.

There are about 26-60 varied species of wild daffodils. They contain toxic sap which is harmful to other flowers.

The Ornatus has been long cultivated in Europe. It originates from France and was one of the earliest daffodils to be cultivated. The Empress was bred by a 'first-generation breeder,' William Backhouse in the UK.

Methodology

DNA Extraction - break apart plant cells using lysis buffer, vortex, heat and centrifuge. Remove unwanted materials. Add buffer, incubate, and centrifuge in spin column with filter. Capture DNA by pipetting liquid into a new spin tube to trap DNA, add buffer, and centrifuge. Transfer into new tube. Add buffer to release DNA and centrifuge. We have DNA!

DNA Preparation, Running DNA Sequencer and Collecting DNA - the DNA library is prepared by cutting the DNA sample using transposase enzyme complex into DNA fragments to increase sequencing efficiency. Transposase adapters are ligated onto ends of fragments by DNA ligase. Sequencing adapters attach at 30° C – contain complementary tether sequence allowing the DNA fragment to link to the nanopore on the protein to help unwind DNA fragment. Heat to 80° C to stop reaction by killing enzyme. Prepare sequencing kit. Prime and load flow cell. Run sequencer and collect data.

Genome visualization, alignment and analysis - gather and drop the sequence data files into one place. Align and colour the bases to highlight differences in the sequences. Analyse the result using the consensus row which measures the similarity in the columns. Calculate a tree - to illustrate how distantly related the genomes are and their common ancestor.

Predicted results

We expect to successfully sequence the chloroplast genome of Ornatus and Empress cultivars. We expect to see high levels of nucleotide sequence similarity in the consensus row but some areas where the nucleotide sequence is different.

Conclusion

We expect to see differences in Ornatus and Empress for the following reasons. Firstly, the two cultivars are derived from different Narcissus sub species (Empress*: N. pseudonarcissus*) and (Ornatus: *N. radiiflorus*). Secondly, they originate from different countries and may have acquired mutations which have been selected for by different climates. Lastly, the two daffodils have different structural phenotypes and so if they appear different it is likely they will have different genotypes too.

Evaluation or next steps

It would be interesting to compare the sequences of Empress and Ornatus to other daffodil species, and closely related taxa such as Allium. The use of molecular clocks would allow us to estimate the approximate time at which they diverged.

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