Are the sequences of the chloroplast genomes of the daffodils *N. radiiflorus var. poetarum* and Princeps different?

Beaulieu Convent School & University of Dundee

Overview

This investigation will use DNA sequencing technology to work out the evolutionary relationships between the *Narcissus radiiflorus var. poetarum* and the Princeps cultivar in order to test the hypothesis that these two daffodils were bred together to create the Lucifer cultivar. We are collaborating with St Peter the Apostle High School and Queen Anne High School to decode the three different daffodil chloroplast genomes.

Aims

We aim to use the DNA within the chloroplasts to assess how closely related two different daffodils are, the *Narcissus radiiflorus var. poetarum* and the Princeps cultivars. We will then be able to construct a phylogenetic tree to show their evolutionary relationships.

Background information

Daffodils have a rich history in the UK. With cultivation thought to have originated in ancient Greece, humans have played a pivotable role in generating over 30,000 named varieties from less than 60 different species. Most of this selective breeding has taken place in the last 200 years but little record of lineage has been made. The chloroplast is a microscopic organelle found within leaves. It is responsible for photosynthesis and is therefore found within the green cells of plants. In addition to the photosynthetic machinery it also contains a small circular ring of DNA. To date there have been only 3 complete 2 chloroplast genomes sequenced from daffodils showing that there is variation within this sequence.

Methodology

Both the *Narcissus radiiflorus var. poetarum* and the Princeps cultivars were supplied as bulbs to the school in October. The bulbs were grown in large pots in general purpose peat-free compost. Both daffodils have both broken through (February) and therefore we intend to perform the following method in March building on the methods we have previously developed.

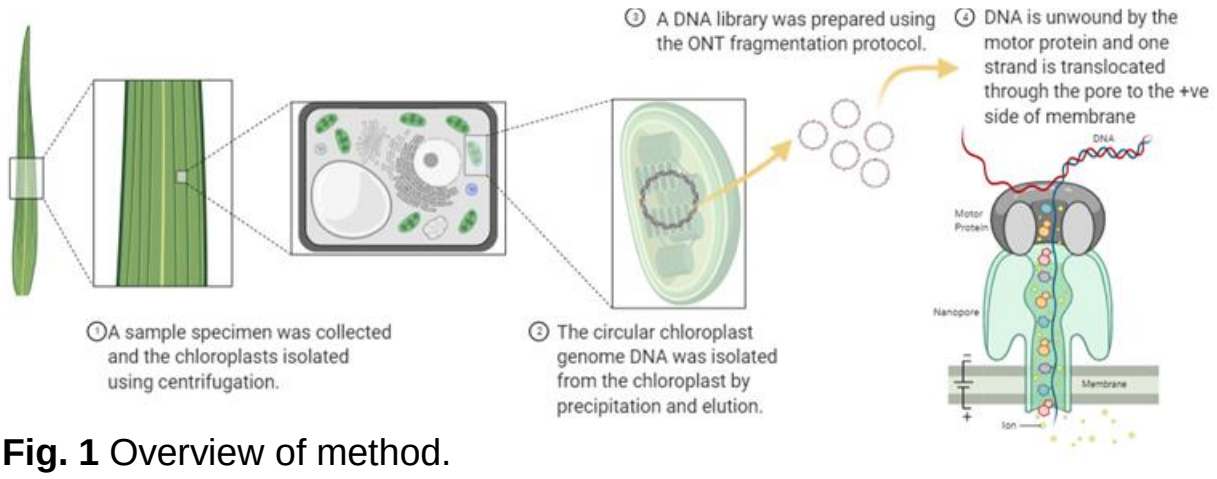

Fig. 1 Overview of method.

1. Destarching the leaves overnight in the dark before macerating in an ice-cold mortar with ice-cold chloroplast isolation buffer (Merck). Destarching prevents chloroplasts from being ruptured during the early centrifugation steps. The resultant macerated suspension is then filtered.
2. Maximising the chloroplast concentration by firstly centrifuging the macerated suspension at a low speed for less than 2 minutes. This creates a white pellet of cell walls and some nuclei. The supernatant is then centrifuged at high speed for 7 minutes resulting in a green pellet of intact chloroplasts.
3. Using the Qiagen Dneasy kit, the DNA is extracted from the chloroplasts, before preparing the DNA for sequencing in the MinION with a Flongle flowcell. The chloroplast genome will then be assembled using previously published sequences with Geneious Prime software.

Predicted results

We have previously shown that the published chloroplast genome for *N. poeticus* is the most appropriate to use as the reference sequence for single flowering daffodils with a single trumpet corona. We believe that the *Narcissus radiiflorus var. poetarum* will be similar to this reference genome with the Princeps more different.

Conclusion

In our previous attempts we have sequenced two naturalized daffodils of unknown origin (one division 4 double daffodil in 2020, and one division 8 bunch-flowered daffodil) and 3 daffodils from the Brodie Castle collection (Coulmony, Morven and Coverack Beauty). We have been able to build the following phylogenetic tree based on our data and that of the three published genomes.

Next steps

We will be starting our focused efforts on the Princeps cultivar as this has the most growth to date. We should have a draft sequence complete within 5 days of the wet lab.

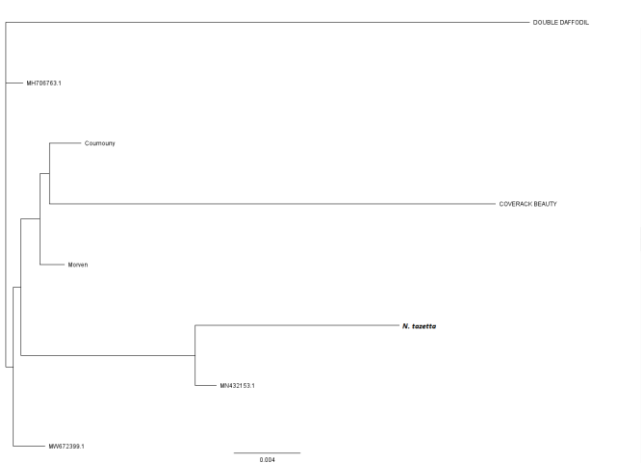

Fig.2 Phylogenetic tree of our previous experiments against published genomes

Fig. 3 *Narcissus radiiflorus var. poetarum (L)* and Princeps (R).

FUNDED BY A PARTNERSHIP GRANT FROM THE ROYAL SOCIETY

Beaulieu Convent School Jersey

Dundee Botanic Garden
University of Dundee