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# Two chromosomal locations of fertility restorers for petaloid CMS in carrots

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## Introduction

- Cytoplasmic male sterility (CMS) is a maternally inherited condition where plants cannot produce their functional pollen due to interactions between mitochondria and specific nuclear genes.
- In carrots (*Daucus carota* L. subsp. *sativus* (Hoffm.) Arcang.), there are two major CMS types: petaloid, where stamens are replaced by petals, and the brown anther type, where anthers become degenerated.
- In both cases, the sterilizing effect can be reversed by nuclear genes known as fertility restorers. These genes interact with the mitochondrial sterility determinants, effectively reversing the sterility phenotype and enabling the production of functional pollen.

## Aim

The objective of this study was to identify the chromosomal location of fertility restorer genes in four distinct carrot populations.

## Materials and methods

Four populations (168, 172, 510-14, 511) were analyzed. These populations segregated into petaloid male-sterile and male-fertile (restored) plants (Table 1). In two populations an occasional appearance of brown anther plants was also noted. All populations were developed in collaboration with Plantico (Zielonki Parcela, Poland). Genotyping was conducted using molecular markers from chromosomes 4 and 9 (Table 2). For DNA amplification both regular PCR and Long PCR were used.

- For regular PCR *Taq* DNA polymerase (DS BIO) was used and for Long PCR – Long PCR Enzyme Mix (Thermo Fisher Scientific).
- Amplification products were electrophoresed in standard agarose gels (SCAR markers) or digested with a restriction enzyme (Table 2) which was followed by electrophoresis in agarose or in a native polyacrylamide gel (CAPS markers).

Table 1: Populations used in the study.

| Population | Number of plants | | | |
|---|---|---|---|---|
| | Total | Fertile | Petaloid | Brown anther |
| 168 | 104 | 26 | 67 | 11 |
| 172 | 81 | 39 | 33 | 9 |
| 510-14 | 46 | 22 | 24 | 0 |
| 511 | 70 | 33 | 37 | 0 |

Table 2: Description of the used markers.

| Name | Type | Chromosomal location | Restriction enzyme |
|---|---|---|---|
| pe098 | CAPS | 9 | RsaI |
| pe106 | CAPS | 9 | TaqI |
| pe957 | CAPS | 9 | CviJI |
| pe880 [1] | CAPS | 9 | CviJI |
| CarPIST [2] | CAPS | 4 | DraI |
| DcS 01 to 12 [3] | SCAR | 4 | - |

[1] for population 172, polymorphism was visible already after PCR – restriction was omitted
[2] marker according to Fujii and Shiroto (2019)
[3] these markers were kindly provided by Dr hab. Alicja Macko-Podgórni, prof. UAK

## Results

In population 168 co-segregation with the phenotype was observed for three markers from chromosome 4. Depending on the used marker co-segregation ranged from 72 to 84 %. In case of population 172 only one marker showed co-segregation (67 %) with the phenotype. That marker originated from chromosome 9. The highest co-segregation value – 98 % was observed for population 510-14 genotyped with another marker from chromosome 9. None of the analyzed markers showed co-segregation with the phenotype in population 511.

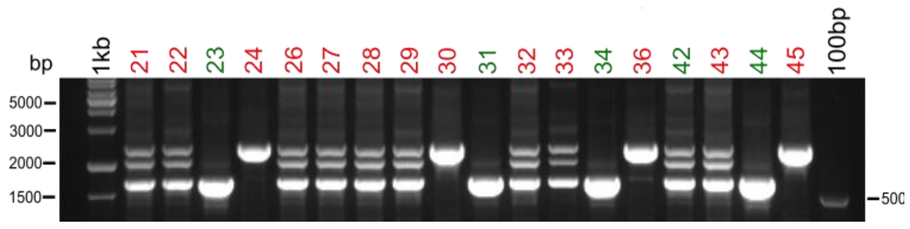


Figure 1: PCR products obtained with marker Chr 04 DcS 04 for selected plants from population 168.

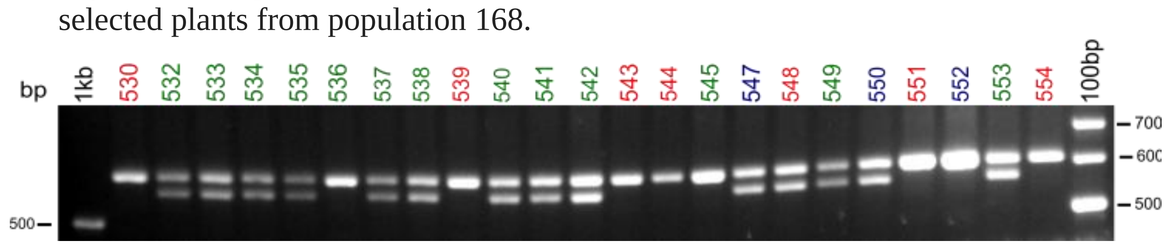


Figure 2: PCR products obtained with marker pe880 for selected plants from population 172.

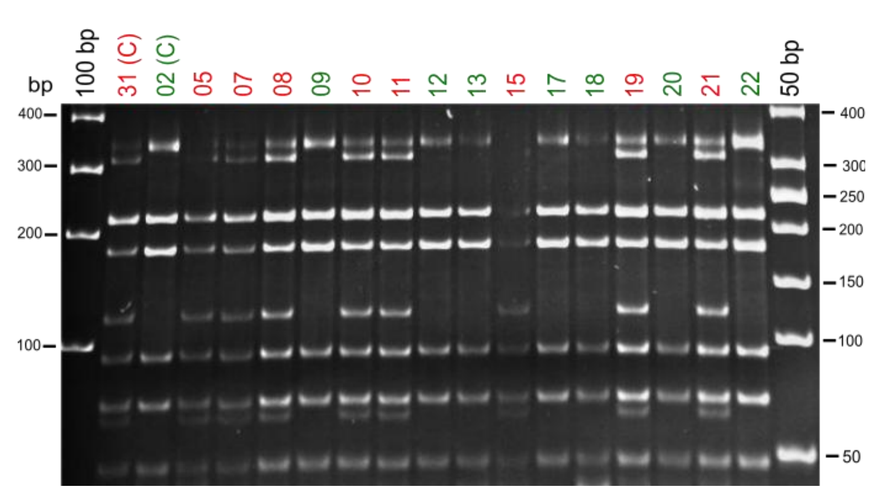


Figure 3: Restriction products obtained with marker pe957 for selected plants from population 510-14.

## Conclusion

The restorer from population 168 is located on chromosome 4
Populations 172 and 510-14 have their restorers on chromosome 9.

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