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Basic genetic characterization of *Adonis* spp. germplasm for breeding and domestication



Key external stakeholders:

Food Ingredients and Functional Foods Sector, Aquaculture Sector, Alternative Crops Sector

Practical implications for stakeholders:

Adonis palaestina is a potential alternative crop, producing a high value compound, the red pigment astaxanthin, with potential nutraceutical and food ingredient applications. We show that the genetic diversity of currently available unadapted germplasm is sufficient for the foundation of a fast-track breeding process, and that small and large flowered variants cannot be hybridised successfully due to divergent genome sizes. The results provide a knowledge framework to proceed with the effective rapid domestication of the species.

Main results:

Genome and taxonomic relationships between small and large flowered variants of *Adonis palaestina*, and the ornamental species *Adonis aestivalis* are poorly understood, and need to be better resolved in order to maximally exploit the available germplasm for domestication of the species for astaxanthin production.

The genetic diversity of currently available germplasm of the large flowered variant of *Adonis palaestina* is sufficiently high to act as a primary gene pool for domestication efforts.

Opportunity / Benefit:

The majority of agricultural crop species have been domesticated through a mixture of unconscious and conscious selection over a millennial timescale. Species that exhibit potential as novel agricultural crops for the production of high value compounds are frequently completely undomesticated, and little may be understood of their reproductive biology. In order to fast-track this domestication process, breeders need to have a solid understanding of several parameters including the taxonomic relationships of the best candidate species/families for domestication and the genetic diversity of the germplasm that will be used as a foundation of future "elite" selected material. The results outlined here provide baseline information that helps to inform the route for domestication of *Adonis* spp.

Collaborating Institutions:

University College Cork

Teagasc project team: Dr. Dan Milbourne (PI)
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1. Project background:

Bright-red flowers of *Adonis palaestina*, a wild plant that originates from the middle-east, are a potential natural source of the red pigment astaxanthin, a carotenoid which is used as both a feed additive for fish farming of salmonid species, and as a nutraceutical due to its strong antioxidant properties. Currently, a programme of accelerated domestication is underway for this species at UCC. While early work on adapting the species for cultivation focused on a small flowered variant with a double row of petals, current work is focusing on a larger flowered variant, because of the increased astaxanthin yield potential of this type relative to the small-flowered type. Due to the lack of successful sexual hybridization between the large and small flowered variety, and other preliminary data, it has been thought that the large flowered variant is a tetraploid form of the small flowered variant. Recent domestication efforts have focused on the use of the large flowered variant, so knowledge of the likely ploidy level of this form, and its taxonomic relationship to the small flowered variant are important characteristics.

In order to develop baseline information to assist in the domestication process, we performed a preliminary assessment of the ploidy/genome size, genetic diversity and relative taxonomic relationships of *Adonis* germplasm being used in the UCC domestication programme.

2. Questions addressed by the project:

Is there sufficient diversity in the germplasm of large flowered *Adonis palaestina* to allow response to selection and to avoid a genetic bottleneck during the domestication process?

What is the relationship between the best candidates for domestication, namely, putative large and small flowered variants of *Adonis palaestina*, and the ornamental species *Adonis aestivalis*.

3. The experimental studies:

Genetic diversity of large flowered *Adonis palaestina*

When initiating a domestication process it is useful to establish the amount of genetic diversity existing in the germplasm upon which the domestication programme will be based, in order to ensure sufficient genetic variation exists to allow response to selection during breeding, and to avoid the eventual generation of adapted germplasm with a narrow genetic base. We tested a subset of lines of the available large-flowered *Adonis* germplasm available at UCC using AFLP analysis.

Genome size and taxonomic relationships of available *Adonis* germplasm

The ploidy of a species is another important factor to establish at the beginning of a process focused on the rapid domestication of a species. Ploidy level (diploid vs auto- or allo-polyploid) affects both the inheritance of traits and factors such as the number of generations required to achieve complete homozygosity in an inbreeding species. As an initial experiment to assess the ploidy of the large-flowered variant of *Adonis*, we used flow cytometry to compare the nuclear DNA content of the 2 variants of *Adonis* and estimate their genome size relative to a species with a known genome size – in this case the oat genome.

Based on the flow-cytometry results, more information relating to the real taxonomic organization of the available germplasm was required. Therefore, PCR primers designed to amplify an approximately 800bp region of the nuclear ribosomal RNA gene (between the 18S and 26S subunits) were applied to 86 accessions of *Adonis*, including 48 of the large flowered variant, 26 of the small flowered variant and 15 accessions of *A. aestivalis*, a small flowered *Adonis* species that has been partially domesticated for use as an ornamental plant (summer pheasants eye).

4. Main results:

Genetic diversity of large flowered *Adonis palaestina*

Pairwise similarity values (based on Jaccard's Index) ranged from 0.195 to 0.449 for the breeding material tested indicating that extensive genetic diversity is present in the germplasm being used as the foundation of the domestication programme in this species.

Genome size and taxonomic relationships of available *Adonis* germplasm

The small flowered variant exhibited a nuclear DNA content similar to that of oat, which has a haploid genome size of 12,961 Mbp. Based on our initial assumption that the large-flowered *Adonis* variant was a tetraploid version of the small-flowered variant, we expected flow cytometry analysis to reveal that the nuclear DNA content in the former was approximately twice that of the latter. However, contrary to expectation, analysis reveals that the nuclear DNA content of the large-flowered variant is significantly smaller (by almost 50%) than that of the small-flowered variant. The results of the flow-cytometry analysis call into question our original assumption of the relative ploidy levels of the two flower-size variants of *Adonis*. The radically divergent genome sizes suggest that either the small flowered variant is actually polyploid relative to the large flowered variant, or that the proposed species relationship between the variants is not correct.

The PCR products generated from the rRNA gene were sequenced and aligned using MEGA 4.1 to reveal numerous single nucleotide polymorphisms between the accessions. Further analysis revealed that the SNP data divide the genotypes into two easily distinguishable subgroups. However, contrary to expectation, the sequence data places the small flowered variant of *Adonis palaestina* and *Adonis aestivalis* in one subgroup, while clearly separating the large flowered variant into another subgroup. The fact that the small flowered variant is more closely related to the ornamental species *A. aestivalis* suggests that the taxonomy proposed for this small group of closely related *Adonis* species is incorrect.

5. Opportunity/Benefit:

The results indicate that the process of domestication underway for the large flowered variant at UCC will not be hampered by a lack of genetic variation in the starting material. Better resolution of the species and genome size relationships between large and small flowered variants of *Adonis* spp. currently being used suggests that large flowered *Adonis* is reproductively isolated from both the small flowered variant, and the species membership of the germplasm should be clarified to identify the appropriate genepool for any future introgression work required. The output of the project has specific benefits to an on-going effort to realise the commercial potential of this species.

6. Dissemination:

Results were disseminated mainly at scientific meetings including the Irish Plant Scientists Association Meeting (IPSAM) and the Agricultural Biotechnology International Conference (ABIC).

7. Compiled by: Dan Milbourne