Research Stimulus Fund

Final Report

Development of a new nutraceutical and pharmaceutical crop for Ireland

DAFM Project Ref No: RSF 05214
Start date: 01/01/06
End date: 31/12/09

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Email: p.jones@ucc.ie

Other Principal Collaborating Researchers: Dr Dan Milbourne, Teagasc

Please tick below the appropriate area on the research continuum where you feel this project fits

<table>
<thead>
<tr>
<th>BASIC/FUNDMENTAL</th>
<th>APPLIED/PRE COMMERCIAL</th>
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<tbody>
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<td>X</td>
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</table>

Key words: (max 4) Adonis, astaxanthin, crop domestication
1. **Rationale for Undertaking the Research**

Tillage agriculture is under pressure in Ireland. Returns from most traditional tillage crops such as cereals in Ireland are low, while research into the development of “new” crops, such as sunflower, is focused on the major crop growing areas of the EU such as France, with a very different climate from that pertaining in Ireland. The rationale behind this research programme was to develop a novel crop where the characteristic Irish growing conditions (mild winters, especially in the maritime regions, mild summers with long summer photoperiod, resulting in a long growing season) were particularly appropriate. This crop would need to have a product for which there was a currently unfulfilled market ideally with a large high-value, low-volume export component, resulting in high returns for Irish farmers and for the country.

Following years of preliminary research in UCC, astaxanthin production from the wild plant *Adonis palaestina* was identified as a suitable candidate for such a project. The red pigment in the flowers of *A. palaestina* was identified as astaxanthin, with the same isomeric composition as found in wild salmon. At the start of the programme, the global market for astaxanthin, the pigment used in the feed of farmed salmon, trout, prawns etc. to achieve the desired flesh pigmentation, was approximately €120 M, and growing steadily, with a high unit price of almost €2K per kg. The market was supplied almost exclusively by the nature-identical synthetic product from Hoffman-LaRoche, although there were commercial programmes to develop natural sources of astaxanthin from microalgae or yeast. Natural astaxanthin could claim a share of the market for the growing organic seafood sector, while it was likely that crop-derived astaxanthin could undercut synthetic astaxanthin on the basis of price.

Research carried out before the programme started by the UCC partner had identified environmental conditions required for maximum astaxanthin yield: free-draining soils, pH 6-8; temperatures no lower than –6°C (frost sensitivity) during the growing season or higher than 30°C (heat sensitivity); long photoperiod (>16 hours/day) during the summer flowering period (to maximise flower size). The mild winters in the coastal regions of Ireland, affected by the Gulf Stream (or North Atlantic Drift) would also open up the possibility of an autumn-sown crop, with higher crop yields than the spring-sown crop.

2. **Research Approach**

For Research Task 1 (RT1), the aim was to develop breeding strategies to accelerate the development of improved *Adonis* lines in order to achieve a domesticated crop in as short a time as possible. This is a novel high-technology approach to use on crops which normally respond well to conventional selection, but the aim here was to determine whether this technology was appropriate here.

To produce a high-density linkage map of *Adonis* chromosomes and to develop diagnostic markers for individual breeding targets, a modification of the F2 bulked segregant analysis method was employed, using microsatellite (SSR) markers to screen fully-phenotyped F2 families from multiple F1 plants (full-sib families). To identify widely diverse parents to generate the F1 plants, a new high-throughput genotyping method was used, employing fluorescently labelled AFLP markers (fAFLP) on an ABI 3100 platform. A novel more efficient method for identifying SSRs, using a SSR-enrichment technique eliminating the need for construction of a genomic library, was employed.

More breeding work was initiated in the programme than was originally envisaged, including heritability studies, induced mutagenesis and induced polyploidy, techniques which only became appropriate from the discovery that the two populations used in the study were both diploid and distinct species.
Research not described in the original approved project proposal was also carried out on the dormancy mechanisms in *Adonis* seeds and strategies for breaking the dormancy in order to achieve shuttle breeding between the northern and southern hemispheres.

Novel methods were also developed for the cardenolide studies, including a novel high-throughput HPTLC separation and visualisation technique for cardenolides in *Adonis* tissues. Identification of individual cardenolides, though not originally envisaged in the project, was also carried out by standard HPLC and NMR.

For Research Task 2 (RT2), standard agronomic techniques, such as nutrients, growth retardants, other agrochemicals, sowing rates and times were used to optimise astaxanthin yield. As *Adonis* had never been grown before on a commercial scale, all of the harvesting and post-harvest techniques were novel.

### 3. Research Achievements

Domestication and improvement of *Adonis* has been achieved using conventional breeding, mutagenesis and induced polyploidy programmes and the foundations for marker-assisted selection in an *Adonis* breeding programme has been established. Mechanical harvesting of *Adonis* flowers was achieved for the first time and a technique for producing a petal-only preparation was developed, resulting in the highest astaxanthin content of any natural source. Techniques were developed for reducing by 99% the content of potentially toxic cardenolides from the astaxanthin extract, so that cardenolide concentrations in fish fed *Adonis* astaxanthin were well within the safe levels, using novel methods developed in UCC for quantifying cardenolide content in fish and for a high-throughput cardenolide characterisation from petal extracts.

Cost-benefit analysis was carried out on the value of growing *Adonis* in Ireland. Astaxanthin produced a maximum net return of more than €1000 per ha. The most cost-effective method of extracting cardenolides from *Adonis* for the herbal medicine market was determined to be extraction of the waste green tissue from flower harvesting, producing a potential net return similar to (and in addition to) that for astaxanthin. A crop of 1000 ha would produce enough astaxanthin to capture 5% of the world market and enough cardenolide to satisfy the entire market for herbal *Adonis* herb. *Adonis*-derived astaxanthin successfully pigmented trout and seabream to commercially acceptable standards. In a comparison with synthetic astaxanthin in Japan, the *Adonis* astaxanthin produced seabream with superior visual, taste and mouthfeel characteristics.

### 4. Impact of the Research

Overall, the results of this research programme indicate that *Adonis* is well suited to cultivation in Ireland, probably more so than other countries as a result of mild winters in maritime regions, where higher-yielding autumn-sown crops would give even higher yields. Net returns to farmers are high, with markets which are growing (astaxanthin for both the aquaculture and human nutraceuticals markets, cardenolides for the herbal medicinal product market).

Natural products like *Adonis*-derived astaxanthin are more acceptable to consumers than synthetic versions, even if nature identical. The characteristics of *Adonis* astaxanthin (natural, same isomer profile as in wild fish) should result in a premium product. Seabream trials identified unexpected but desirable advantages over fish fed astaxanthin.
A rapid HPTLC method was developed as part of this programme which enables regulators to identify farmed fish which have been pigmented with Adonis-derived astaxanthin. Helping the policing of sales by regulators.

*Adonis* is a crop with little impact on the environment, as a result of its low dependence on agrochemicals.

5. **Exploitation of the Research**

The research has shown that Adonis is a crop well-suited to Irish tillage farming and climatic conditions, generating high returns for growers, from a high-value product (astaxanthin) for the fish feed, food colorant and human nutraceuticals market, with the possibility of a second moderate-value product (cardenolides) for the herbal medicine market. Astaxanthin derived from Adonis has been shown to pigment several commercial fin and shellfish to levels acceptable to consumers and producers and has proved to be superior to the industrial standard (synthetic astaxanthin) in terms of bioavailability, and similarity to natural astaxanthin.

6. **Summary of Research Outputs**

(a) Intellectual Property applications/licences/patents
1. 2.

(b) Innovations adopted by industry
1. The Irish company Cybercolors is selling Adonis-derived astaxanthin from crops grown in China
2.

(c) Number of companies in receipt of information
Two (Cybercolors (Ireland), Sinotrade (China)

(d) Outcomes with economic potential
1. *Adonis* would provide net returns greater than the most valuable current crop in Ireland, potato

2. There is potential for both import substitution and exports of astaxanthin and cardenolides from *Adonis* grown in Ireland.

(e) Outcomes with national/policy/social/environmental potential
1. Our research has shown *Adonis* to be a crop with little dependence on agrochemicals (reduced pollution problems and which is peculiarly well-suited to Irish conditions, with astaxanthin yields twice those achieved in China. Preliminary studies suggest that maritime sites in Ireland would allow *Adonis* to be autumn sown in Ireland, further increasing astaxanthin yields. *Adonis* is a renewable source of astaxanthin, unlike synthetic astaxanthin
2.

(f) Peer-reviewed publications, International Journal/Book chapters.
1. Guiney, K., Foley, L.M. and Jones, P.W. Studies on the pigmentation of fish using astaxanthin oleoresin extracted from *Adonis palaestina* flowers. Submitted to *Aquaculture*


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7. **Permanent Researchers**

<table>
<thead>
<tr>
<th>Institution Name</th>
<th>Number of Permanent staff contributing to project</th>
<th>Total Time contribution (months)</th>
<th>Average time contribution per permanent staff member</th>
</tr>
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<tbody>
<tr>
<td>UCC</td>
<td>1</td>
<td>4.8 man-months</td>
<td>1.2 man-months p.a.</td>
</tr>
<tr>
<td>Teagasc</td>
<td>1</td>
<td>2.52 man-months</td>
<td>0.84 man-months p.a.</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>2</strong></td>
<td><strong>7.32 man-months</strong></td>
<td><strong>2.04 man-months/year p.a.</strong></td>
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</tbody>
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8. **Researchers Funded by RSF**

<table>
<thead>
<tr>
<th>Type of Researcher</th>
<th>Number</th>
<th>Total Time contribution (months)</th>
<th>Average time</th>
</tr>
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<tbody>
<tr>
<td>Post Doctorates</td>
<td>1</td>
<td>36</td>
<td>12 man-months p.a.</td>
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<tr>
<td>Contract Researchers</td>
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<td></td>
<td></td>
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<tr>
<td>PhD postgraduates</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Masters postgraduates</td>
<td>1</td>
<td>36</td>
<td>12 man-months p.a.</td>
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<tr>
<td>Temporary researcher</td>
<td>8</td>
<td>47.76</td>
<td>2.04 man-months/person/year</td>
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<tr>
<td>Other</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>10</strong></td>
<td><strong>119.76</strong></td>
<td><strong>26.04</strong></td>
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9. Postgraduate Research

Total Number of PhD theses: ___

Total Number of Masters theses: 1

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date: OCTOBER 2012
Gianluigi Bigio. Investigations into the genetic improvement of *Adonis* spp. University College Cork

10. Project Expenditure

Total expenditure of the project: €414,520.52

Total Award by RSF €414,151.

Other sources of funding (specify) €
1. 
2. 

Breakdown of Total Expenditure

<table>
<thead>
<tr>
<th>Category</th>
<th>Name UCC</th>
<th>Name Teagasc</th>
<th>Name Institution 3</th>
<th>Name Institution 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contract staff</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporary staff</td>
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<tr>
<td>Post doctorates</td>
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<tr>
<td>Post graduates</td>
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<td>€61,911.09</td>
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<td>Consumables</td>
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<td>€53,448.00</td>
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<td>Travel and subsistence</td>
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<td>€8,146.16</td>
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<td>€309,257.21</td>
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<td>Durable equipment</td>
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<td>Other</td>
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<td>Overheads</td>
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<td>€95,573.31</td>
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<tr>
<td>Total</td>
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<td>€114,400.30</td>
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<td>€414,520.52</td>
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11. **Future Strategies**

Further work is hamstrung by a lack of funding to continue this research. The UCC PI continues the breeding programme on an unfunded one-person basis, so that progress is slow but continuing.

12. **Industry Collaboration**

New Zealand Agriseeds, located just outside Christchurch, were sub-contracted to do breeding and agronomic work including harvesting studies during the winter period in Cork, so that progress was rapid.

Sinotrade, an Anglo-Chinese company, have grown the crop in Mongolia and supplied the large quantities of astaxanthiun needed to carry out the fish trials which have piqued commercial interest in the product, particularly in Japanese aquaculture sector.