Renewable Materials
LINK Programme

Project Summary

ARTEMISIA - Developing an alternative UK industrial crop *Artemisia annua*, for the extraction of Artemisinin to treat multi-drug resistant malaria

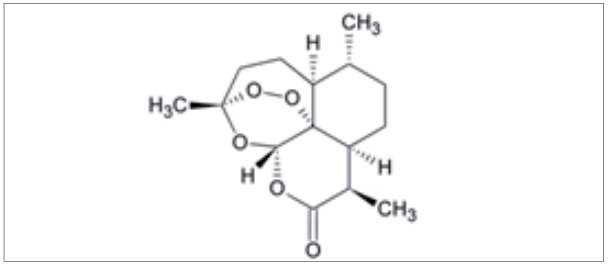
This Factsheet outlines the results of a research project funded under the Renewable Materials LINK Programme.

Background

Malaria, is one of the most serious diseases to beset humans worldwide. The causative parasite, *Plasmodium sp.*, attacks red blood cells causing at best, debilitating, recurring fever and at worst, death especially in the old, the young and pregnant women. Originally, quinine was extracted from the bark of the Cinchona tree and was used to kill the parasite. Subsequently, many highly successful synthetic chemicals were developed from quinine. Unfortunately, the parasite has developed resistance to these medicines, but new products have now been developed using a different plant-derived compound called artemisinin, which is extracted from *Artemisia annua*.

Artemisinin is a complex secondary plant metabolite, which cannot be economically synthesised *de novo*. The unique configuration of the oxygen atoms in this molecule are key to its potent anti-malarial activity. However, this makes it difficult to synthesise.

The WHO has estimated that by 2012, there will be 400 million people suffering from malaria and they will need around 200 tonnes of artemisinin per year. The supply of artemisinin is a critical part of the provision of combination therapy for the treatment of chloroquine-resistant malaria and is likely to remain so for the foreseeable future. In view of this gloomy prediction, we formed a consortium to look at the feasibility of growing *A. annua* in the UK for production of artemisinin-based therapies to serve those regions where malaria remains a problem.



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Collaborators: NIAB; East Malling Research; De Montfort University; Frontier Agriculture; Humber VHB; Horticultural Development Council (HDC)

Funder: Defra

Growing A. annua in the UK.

UK agriculture practice is amongst the most efficient and innovative in the world. Therefore the consortia believe UK production of artemisinin can act as a baseline supply of this vital active. Thus stabilising the peaks and troughs of global production (ie supply) and income to small third world farmers.

Flowering in *A. annua* is triggered by short days. In England this occurs at the end of the summer. In equatorial regions where day length remains more or less the same, flowering can be triggered when the plant has reached a minimum level of maturity. Since artemisinin is extracted from leaf material, cessation of leafy biomass production at the onset of flowering potentially limits further useful harvest.

A second consideration is the total amount of artemisinin that can be extracted from the leaves. In several parts of

A. annua

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Africa, *A. annua* plant lines, that yield well elsewhere, have been found to yield disappointing levels of the active. Some evidence suggests this may be due to the imbalance of minerals in the soil, especially iron compounds.

Finally, artemisinin can be lost through degradation following harvest if it is not extracted and stored in a stable form. Considerable losses, in terms of activity, have been documented during the transport of material to sites producing the finished medicines.

Challenges in developing the crop

Since this was effectively a completely new crop for the UK, we considered it vital to address, as far as possible, all parts of the value chain. Without this approach, there would be a danger that growers would be in an impossible position in terms of sourcing reliable seed, identifying whether the artemisinin was present in their crop and finally, selling on to companies producing the finished product. We considered agronomic, genetic, biochemical and plant physiological issues relating to its cultivation in the UK.

Initially, seed was collected from botanic gardens, private collections and commercial sources. The seed is tiny at circa 10- 20,000 seeds per gramme and the negative consequences of this are many. Low seed quality and poor germination was an issue. During the early stages of establishment, the small, delicate seedlings were susceptible to competition from weeds, while also being sensitive to the herbicides used to control those weeds.

When a crop was drilled mechanically, precision was difficult as the seeds were very sensitive to drilling depth, with poor germination below a few millimetres. Transplanting greenhouse-reared seedlings was an option, but it increased costs considerably.

Finally, the increase of the total artemisinin in the foliage using classic plant breeding approaches was investigated. As an outcrossing species, we encountered problems relating to inbreeding suppression. The result of this is that crosses between very closely related lines yield plants with poor vigour and very low seed production.

By addressing these considerations, we have successfully increased seed quality, which are larger, have improved germination rates and yield plants that are more closely synchronised in terms of flowering dates.

Worldwide success

So far, the artemisinin concentration has been improved by nearly 200% over commercial lines and our best lines are yielding at least 2.2% w/w dry material. This is undoubtedly the best germplasm in the world in terms of artemisinin content and this result has been verified by external laboratories. Furthermore, significant improvements have been made to the extraction and clean-up efficiency. We have also sought to match this with improvements in health and vigour of the plant. Trials of herbicides are ongoing with some success, so far, at post emergence, but there have been considerable differences between years. Over 2,500 samples have been chemically profiled both from new, developed lines of *A. annua* and from original parent lines and commercial 'varieties'. Methods for cheap, efficient assay of artemisinin and high precision determination of artemisinin have been developed. Ultimately, we aim to produce a manual detailing the botany and agronomic specification for cultivation of this new crop in the UK so that growers will be prepared and will consider growing *A. annua* in the quantities that are required.

Exploitation

A sample of seed was grown on an associate farm in Morocco in 2008. Samples have been despatched to major players in Africa and Canada in spring of 2009 and to China, Madagascar, India and Africa in autumn 2009. It is not known how this material will perform in differing environments although the first indications have been favourable. It is also anticipated that considerable trialling may be necessary for these producers to build confidence in the consortium as a seed supplier.

Further Work with Industrial Partners

The Partners wish to continue the collaboration to further improve the germplasm as well as to evaluate various precursor and related compounds extracted together with artemisinin. These compounds are known to have anti-plasmodial and other cytotoxic activities, either alone or in combination. They are present in significant quantities in *A. annua* together with artemisinin and can potentially yield very useful additional value to the crop. It is important that they are recoverable without interfering with the artemisinin extraction process. Further optimisation of seed establishment and harvesting times is needed to bring harvest dates forward. This will help to increase yields of artemisinin, while helping to fit in with other crops in a rotation. Optimisation of plant drying methods will also help to maximise retention of artemisinin levels while reducing the costs. These developments will seek to capitalise on some important know-how gained during the Link programme and relate to combinations of plant maturity and environmental conditions that are conducive to maximal artemisinin content.

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