New hybrid varieties of *Artemisia annua* from the University of York

The CNAP Artemisia Research Project

Tackling malaria with fast track plant breeding

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BIOLOGY TO BENEFIT SOCIETY

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The CNAP Artemisia Research Project

This project is using the latest genetic and analytical technologies to rapidly improve supplies of a vital malaria drug. With the help of molecular plant breeding, we are developing higher yielding, more robust varieties of the world's most important medicinal herb: *Artemisia annua*. Artemisia is the source of the most effective cure for malaria and the new varieties are urgently needed to help meet increasing demand for affordable malaria medicines.

We are using molecular plant breeding and not GM to create these new varieties.

"Their results are innovative in terms of the scale of the research and sophistication of the technologies involved."*

*"The botanical solution for malaria," W.K. Milhous and O.J. Weina, Science 327. no. 5963, pp. 279 - 280 (2010).

The research is based in the Centre for Novel Agricultural Products (CNAP) at the University of York, UK where Professor Dianna Bowles and Professor Ian Graham lead a project team of around 30 molecular biologists, geneticists, biochemists, plant breeders and horticulturalists. The scientists have state-of-the-art facilities for chemical analyses, molecular genetics and bioinformatics. The project is funded by the Bill & Melinda Gates Foundation.

Context

Malaria control

Malaria is one of the world's most serious public health problems, claiming almost a million lives a year every year and undermining development in some of the world's poorest countries. However, with new tools and increased malaria funding, there is now real hope of making progress against this disease and possibly even eradicating it. A massive scaling up of control efforts is underway, and this includes increased use of artemisinin combination therapies (ACTs) to cure malaria.

Artemisinin Combination Therapies (ACTs)

ACTs are currently the most effective cure for malaria. Their active ingredient is artemisinin, which is extracted from the plant *Artemisia annua*. Demand for ACTs is predicted to increase dramatically, from around 100 million treatments in 2008 to 250 million by 2015. However, the low yield of Artemisia creates problems for production and there are concerns that artemisinin supplies will not reliably keep pace with increasing demand for ACTs.

Agricultural production of Artemisia

Artemisia is farmed in Asia and Africa, where it is an important income source for tens of thousands of small-scale growers. Production in these regions creates a year-round supply and enables malarial countries to contribute to their own national health.

However, relatively little has been done to develop the Artemisia plant from a weed into an agricultural crop. Yields are extremely low, and this makes agricultural production of artemisinin expensive and often uneconomic. Artemisia farmers badly need robust higher-yielding varieties.

Although synthetic methods of production are being developed, plant production is expected to remain an essential source of supply.

Benefits from the new varieties

High-yielding Artemisia varieties from this project will bring a range of benefits, helping to;

Stabilise artemisinin supplies

farmers will be encouraged to grow more Artemisia, which will prevent shortage-driven price hikes.

Lower production costs

increasing the artemisinin content of the plant substantially reduces the costs of both cultivation and extraction.

Support small-scale farmers in less developed regions

by providing them with a more robust and profitable crop.

Reduce environmental impacts

by lowering the requirements for transport and the amount of solvent needed for extraction.

Support production of high quality ACTs

seed for the new varieties will be available for Artemisia growers supplying high quality ACTs.

Contribute to increased efforts to tackle malaria

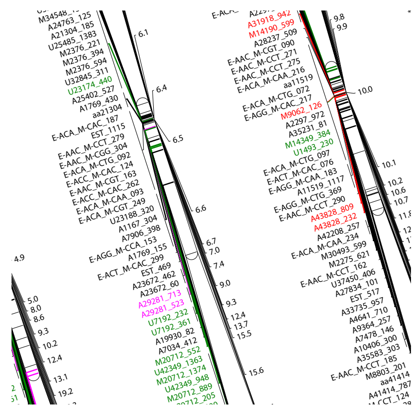
the first high yielding seeds will be available in time to supply increasing demand for ACTs.

Molecular tools for faster plant breeding

We have developed DNA-based techniques for the early stage recognition of promising Artemisia plants. To do this, we identified variations in the plant's DNA that are consistently linked to certain plant features. These DNA sequences may represent genes for a given plant feature, or they may be molecular markers: sequences that lie close to the gene and are usually inherited with it. Molecular markers are reliable DNA indicators that a plant has inherited the associated feature. Some important plant traits are controlled by more than one gene. For these traits, we have identified which regions of the genome are the most important to inheritance: quantitative trait loci (QTL).

In 2010, we published the first genetic map of *Artemisia annua* in Science*. The map plots the relative position along the plant's DNA of around 700 molecular markers and 50 genes, as well as QTLs for key traits. This work represents a major advance in our understanding of Artemisia genetics. It means that valuable Artemisia plants can now be recognised from their DNA at an early stage and makes it possible to breed improved varieties in a fraction of the time it would otherwise take.

* The genetic map of *Artemisia annua* L. identifies multiple trait loci affecting yield of the antimalarial drug artemisinin." Ian A. Graham, Katrin Besser, Susan Blumer, Caroline A. Branigan, Tomasz Czechowski, Luisa Elias, Inna Guterman, David Harvey, Peter G. Isaac, Awais M. Khan, Tony R. Larson, Yi Li, Tanya Pawson, Teresa Penfield, Anne M. Rae, Deborah A. Rathbone, Sonja Reid, Joe Ross, Margaret F. Smallwood, Vincent Segura, Theresa Townsend, Darshna Vyas, Thilo Winzer, Dianna Bowles. Science 327, no. 5963, pp. 328 - 331 (2010).



A pipeline of potential

We select the most promising individuals for our plant breeding with a series of specially-developed tests. Plants are chosen on the basis of features such as metabolite profile and, plant biomass. They are also selected according to their genetics, using our new genetic map. For example, we choose plants with positive genetic scores for key traits such as leaf area and artemisinin concentration. Over 32,000 plants have been assessed in this way, generating a collection of potential high performers. These plants are crossed, using a variety of breeding strategies, to produce experimental hybrid plants. Each stage of this process is guided by further trait and DNA assessments. As the project progresses, more data becomes available to inform each choice. Thus, a pipeline of increasingly high-performing new plant lines is in operation.

Field testing

The best plant lines are selected for further development on the basis of extensive field trials in the commercial production centres of Africa and Asia. These tests ensure that the varieties we develop will perform well when grown under regional agricultural practises. Plants are assessed for their yield, for their robustness and for their resistance to pests and diseases.

Our partners for this work are local artemisinin-producing companies, chosen for their extensive grower networks and their links to the ACT supply chain. The Swiss not-for-profit organisation, Mediplant is also field- testing our plants. Early results have demonstrated that our best plant lines yield significantly more than the current market leader.

field trial sites

User tests

We are producing several new varieties of Artemisia. All will be significantly higher yielding than the current market leader (Artemis) and non GM. In order for our new varieties to make an immediate contribution to malaria control efforts, they must be readily adopted by industry.

To this end, we subject high-performing plant lines to user tests by Artemisia processors and ACT manufacturers. These tests are designed to ensure that our new varieties are compatible with current manufacturing processes. For example, leaf material from large-scale field experiments is sent for commercial pilot extraction to test the artemisinin yield and purity of extracts from our plant lines.

A long-term legacy from the project

In 2011, we are planning to supply seed of our first stage new hybrids for demonstration purposes. Large-scale distribution of hybrid seed will be ready for the 2012 growing seasons. This timing will enable us to supply the predicted peak in demand generated by the scaling up of malaria control measures. Conservation of the project's germplasm will be maintained over the long term in tissue culture at Mediplant. The artemisinin from the new varieties will contribute to supply chains for the manufacture of high quality ACTs.

In this way, the project will make a lasting contribution to improved artemisinin supplies and help to secure better access to effective malaria medicines. We are also working to maximise the benefits to Artemisia growers in developing countries. The Syngenta Foundation for Sustainable Agriculture and Mediplant are working with us to ensure such growers have the knowledge they need to get the best out of the new varieties.

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