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DFID Department for International Development
Plant Sciences Research Programme

Genetic transformation for nematode resistance in rice, potato and cooking bananas for developing countries

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SUMMARY

Nematode-resistant potato, rice and banana are being developed for subsistence growers. Nematodes cause estimated global losses of 20% to banana, 12% to potato and 10% to rice. Higher values are probable in subsistence agriculture. In Africa, using FAO estimates of yields, losses due to nematodes are likely to exceed 6 million tonnes (m t) for banana, 1.5 m t for potato and 1 m t for rice. In Asia, losses are comparable to Africa for banana, and much higher for rice and for potato (15 m t).

A transgenic approach is being used because traditional breeding for nematode resistance is difficult. Transgenic approaches can be faster because breeding is a very long-term process in potato and breeding bananas (a clonal crop) is extremely difficult. The three target crops are all damaged by several different nematode species and each requires a separate and considerable breeding effort. Genetic modification allows the development of plants resistant to a range of nematodes so, for their effective use, growers do not need to know which of these pests are problems.

The genetic modification is highly biosafe as it involves transferring genes from maize or rice that express a protein (cystatin). The cystatin is found in the grain so it is already consumed by billions of humans as part of their diet. The cystatin prevents the nematodes from digesting proteins so they are malnourished, develop slowly and reproduce much less.

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Bringing the benefits to farmers

The most advanced research is in potato, with successful field trials in the UK. However, until now transgenic potatoes from this research have not been tested in developing countries. Minitubers of transgenic potato cultivars Desireé and Revolución have been prepared for despatch to collaborating research institutes in both Argentina and China. The search continues to identify research institutes that wish to test this promising technology in other developing countries with functioning national biosafety regulations in place. A continuing supply of tubers is produced to be able to supply them at short notice at the correct physiological age.

Improving the transgenic strategy in potato

Switching on genes in the right place. To make the genetic modification even more biosafe, transgenes have been developed which only express the cystatin in modified plant cells induced by nematode infestation. This strategy works for species of root knot nematode (*Meloidogyne*) that are a major constraint on developing world agriculture. In other species, such as *Pratylenchus* that is second only to *Meloidogyne* in its importance as a nematode pest, a different strategy is needed because they do not induce changes in host plant cells. The part of the gene that determines when and where a gene is expressed in a plant is its promoter. A promoter that causes expression in every cell including the harvested parts is not the most desirable. Even though the cystatin is proven to be safe for human consumption, consumers may prefer a variety where the gene is not expressed in the part of the plant they eat. A promoter from pea has been identified that has a high expression in the potato roots only, ideal for a strategy for resistance to *Pratylenchus*. It is also likely to be suitable for Pratylenchus in banana and plantains as well as species of other nematodes, *Radopholus* and *Helicotylenchus*, that damage this crop.

Improving the transgenic strategy in potato

Producing more effective genes. Much of the earlier work has used a cystatin from rice. However, maize cystatin has proved to be more effective against nematodes. Cereals tend to have a different codon usage from potato i.e., different DNA sequences that code for the same amino acid. The maize cystatin has been altered to potato codon usage to provide an identical protein with exactly the same amino acid sequence. Use of an appropriate codon usage in potato should lead to higher gene expression levels that should enhance efficacy against nematodes.

Producing alternative resistance mechanisms. The cystatin approach allows the nematodes to infest the plant but dramatically reduces the rate at which they grow and reproduce. An alternative strategy is to prevent the infestation in the first place. Plants have been made that produce a peptide (a small chain of amino acids that can have important biological activity) that stops the nematode from locating and invading the roots. Using this strategy in potato high levels of resistance (70% resistance i.e., only 30% of the infestation of the non transgenic control) has been achieved. Even higher levels of resistance occur to a cyst nematode that is an important pest of potato and rice. The work is now being extended to a Ugandan population of *Radopholus* similis, the most important nematode pest of banana. This demonstrates how outputs flow readily from demonstration of resistance on one crop, usually potato, to other important targets such as banana. This speeds progress by limiting the more difficult transformation in banana to the most promising approaches.

Environmental biosafety of transgenic, nematode resistant potato. The University of Leeds has published a major paper on the biosafety of transgenic potato that might be deployed in Centres of Biodiversity for this crop (Nature 432: 222-225). This was collaborative work between PSP-funded research and an EU INCO project. It established that transgenic potato varieties had less influence on soil micro-organisms than arises from a grower's free choice on what crop species they grow. There are bigger differences between alternative crops such as bean, lupin or potato than between transgenic or non-transgenic. Moreover, there is even a bigger difference between potato varieties than between transgenic and non-transgenic potato.

The work also established that cultivated potato crosses with its wild relatives when they are grown in close proximity. However, it is not yet known if this results in stable introgression of genes from the cultivated to the wild species. The PSP work demonstrated that a South American cultivar, Revolución, that is naturally male sterile can be transformed and nematode resistance achieved. We propose in the Nature paper that this male sterile potato be used for transgenic trials until the risk of introgression of transgenes to wild relatives is determined. In addition, in PSP-funded research we have shown that we can use a transgenic approach to make normally male-fertile cultivars sterile. This work has been accepted for publication. The work is already informing the safe uptake of other traits such as transgenic, insect resistant potato for subsistence growers.

Rice: Demonstrating stably expressed nematode resistant rice

Nematode resistant rice has been developed but the levels of resistance declined through generations of seed multiplication. This is probably due to the method of transformation that was used. This has been changed in a system where the gene for antibiotic resistance, usually used in generating transgenic plants, has been eliminated. A large set of transgenic lines has been produced by this new method and they are currently being screened for nematode resistance.

Banana: East African Highland banana transformation

The first demonstration of an East African Highland banana transgenic for any trait was achieved and established that cystatin confers resistance to Radopholus similis in banana (Fig. 1). Although the resistance is only moderate the lines demonstrate that our goal is achievable of controlling the five major nematode pests of banana without the grower needing to know which of the species has infested the land. We have already demonstrated effective control of four of these nematodes on other crops. The achievement of some resistance in only two lines is very encouraging given that it is planned to generate over 100 lines for each transgene of interest to ensure lines are generated with high levels of resistance for field trials. Progress will be rapid if time is available for further banana transformation and evaluation.

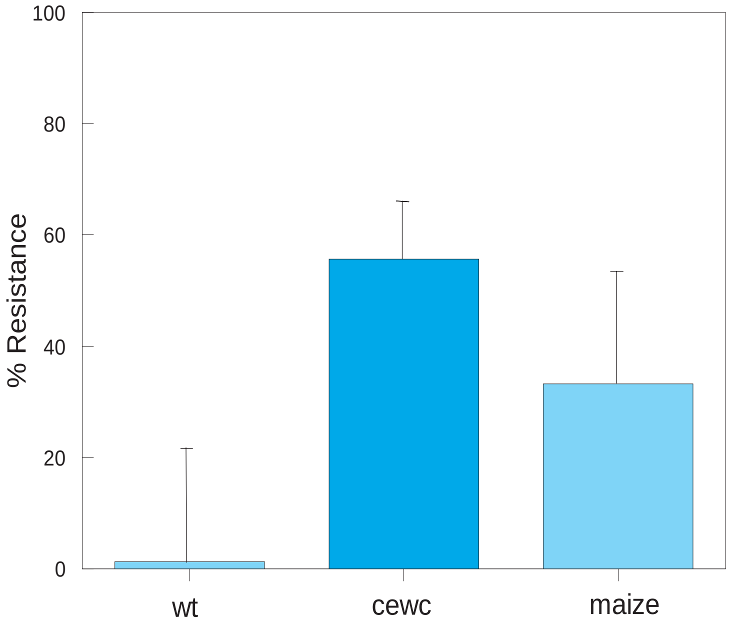

***Fig. 1*. Partial resistance of the only two transgenic East African Highland banana lines available for bioassay. They express the cystatins maize and CEWC.**

Acknowledgements

The work reported here are outputs form a series of research grants to the Centre for Plant Sciences, University of Leeds from the Plant Science Research Programme of the UK's Department for International Development (DFID). The views expressed are not necessarily those of the DFID.