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Genetic transformation *of pigeon pea and sorghum:* creation and field assessment *of pest-resistant transgenic plants*

The two projects developed with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and CIRAD were aimed at controlling insect pests of two important crops in semiarid tropical regions—pigeon pea (legume) and sorghum (grass).

Sorghum is a staple food for people living in semiarid tropical areas and this plant is especially susceptible to pest attacks. The lepidopteran *Chilo partellus,* whose infestation rate sometimes reaches 100%, is one of the most serious pests—destroying sorghum stems and thus keeping plants from reaching the flowering stage, along with many other grasses.

This stem borer is hard to control via pesticide treatments since it lives within the plant. There is no known resistance within the diverse range of sorghum varieties, so a transgenic approach would seem especially well adapted to controlling this pest.

Pigeon peas are grown in many countries and represent an important dietary source of protein. *Helicoverpa armigera* is a polyphagous insect that is active year round and causes major damage to both pigeon pea and cotton crops. Intensive chemical treatments are conducted to control this pest, especially on cotton, but the treatment efficiency is gradually declining.

Misuse of pesticides eliminates natural enemies and gives rise to resistance mechanisms in the pest species. New control methods are being developed or investigated to deal with these problems. These integrated pest management

▷▷ Visiting scientists

▪ SORGHUM PROJECT

Nadoor Seetharama visited France for 4 months in 2001 and prepared molecular constructs for *Bacillus thuringiensis* gene transfer into sorghum.

Jean-Michel Vassal, Isabelle Pieretti and Monique Royer (CIRAD) visited ICRISAT for 1 week (August 2002) with CIRAD and ICRISAT support.

▪ PIGEON PEA PROJECT

Lavanya Madakasira (ICRISAT) visited France for 6 months (September 2002 to February 2003) to construct the synthetic *cry2Aa* gene and prepare molecular constructs for pigeon pea genetic transformation.

Kiran Sharma (ICRISAT) visited France for 2 weeks (December 2003) to assess the project research progress and prospects.

Due to restructuring of ICRISAT during the project, all visits to France that had been planned for Indian researchers could not take place and some experiments thus had to be conducted at ICRISAT in India.

Pigeon pea

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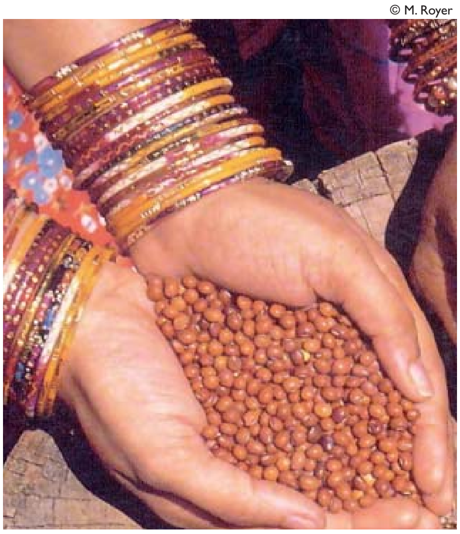

Pigeon peas *(Cajanus cajan)* are being cropped to an increasing extend in some 50 countries on the Indian continent, Southeast Asia, East Africa (Kenya, Tanzania, Uganda, Malawi, etc.) and the West Indies (Dominican Republic, Puerto Rico, Guadeloupe).

This legume represents an important dietary source of protein (20-22% protein content in the peas and leaves). It is often cropped in rotation with cereals. It is also grown on tiny plots in subsistence farming conditions (Cape Verde, Benin). Moreover, it is often used as mulch and, more recently, as fodder. The total area under pigeon pea crops in India is over 4 million ha, but there can be substantial crop losses caused by the pod-infesting *Helicoverpa armigera* pest.

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Sorghum crop field. Inset, a close-up view of an attack by the lepidopteran Chilo partellus *on an adult sorghum plant*

strategies are mainly aimed at reducing chemical pesticide use, while ensuring efficient pest control and safeguarding the regulating action of beneficial parasites and predators.

Transgenic cotton varieties resistant to *H. armigera* and *Heliothis virescens* (a lepidopteran closely related to *H. armigera* but found only on the American continent) were thus first marketed in 1996 and are already cropped in USA, India, China, Africa (Burkina Faso, South Africa), Argentina, Australia, etc.

Research was launched on sorghum and pigeon pea at ICRISAT to develop and field assess plants that have been genetically modified by artificial insertion of 'pesticide' genes coding for toxins produced by the bacterium *Bacillus thuringiensis* (*cf. GMOs to the rescue of agriculture in the South?* p. 35).

The lepidopteran* sorghum stem borer Chilo partellus *is hard* to control with pesticide *spray treatments

The aims of the 'Sorghum project' were to develop transgenic varieties resistant to the lepidopteran *C. partellus* and assess them in the field.

CIRAD provided ICRISAT with access to a set of molecular tools that had been prepared within the framework of a European project geared towards developing transgenic rice varieties resistant to *Chilo suppressalis* (a lepidopteran closely related to *Chilo partellus*). These tools facilitate the transfer of four *B. thuringiensis* toxin genes (*cry1Aa, cry1Ab, cry1Ac* and *cry1B*) into monocotyledon plants. •••

Partnerships

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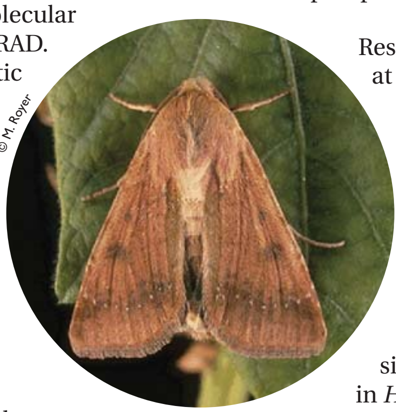
A first generation of transgenic sorghum varieties was obtained by the microparticle bombardment technique using a construct in which the *cry1Ac* gene is under control of a 'wound-inducible' promoter. This promoter induces expression of the gene only when the plant is attacked by an insect, thus reducing toxin production. These *Chilo partellus*-resistant plants have already been transferred to greenhouse conditions.

A second generation of transgenic sorghum varieties was obtained by the genetic transformation (transgenesis) technique via the bacterium *Agrobacterium tumefaciens* using molecular tools developed at CIRAD. In these tools, synthetic *B. thuringiensis* toxin genes *cry1Aa* and *cry1B* are under control of a 'constitutive' promoter (that induces expression of the gene in all plant tissues continuously over time) or a wound-inducible promoter. Plants showing resistance to *C. partellus* were characterized by molecular analysis (DNA analysis and toxin quantification). The T3 generation of these plants is currently being assessed in the greenhouse.

The lepidopteran* Helicoverpa armigera *is a polyphagous pigeon pea pest that is active year round

The main aim of the 'Pigeon pea project' was to develop new transgenic varieties that are resistant to the lepidopteran *H. armigera.*



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Research carried out at CIRAD led to the identification of four *B. thuringiensis* toxins active against *H. armigera,* i.e. *Cry1Aa, Cry1Ab, Cry1Ac* and *Cry2Aa.* Studies on the receptor sides of these toxins in *H. armigera* larvae highlighted the specific behaviour of the *Cry2Aa* toxin and *[Cry1Ac + Cry2Aa]* was found to be the most promising combination with respect to delaying or reducing the risk of resistance development.

A first generation of molecular constructs was prepared at CIRAD. These can be used to transfer *cry1Aa, cry1Ab* and *cry1Ac* genes via *Agrobacterium tumefaciens* under control of specific promoters (promoter of the CaMV virus 35S gene or of the *Arabidopsis thaliana EF1*-α gene). These constructs were taken to ICRISAT and different pigeon pea plants expressing *B. thuringiensis* toxins were obtained in the T0, T1 and T2 generations. These transgenic plants were transferred to the greenhouse, assessed for their resistance to *H. armigera* and characterized by molecular analysis (DNA analysis and toxin quantification).

A new synthetic gene coding for the *Cry2Aa* toxin was also prepared at CIRAD, along with a second generation of constructs that facilitate the transfer of this gene in combination with *cry1Ac*.

Genetic transformation of the pigeon pea with this second generation of constructs was scheduled at ICRISAT for 2006.

▲ *Adult female* **Helicoverpa armigera,** *an insect pest of pigeon pea crops*

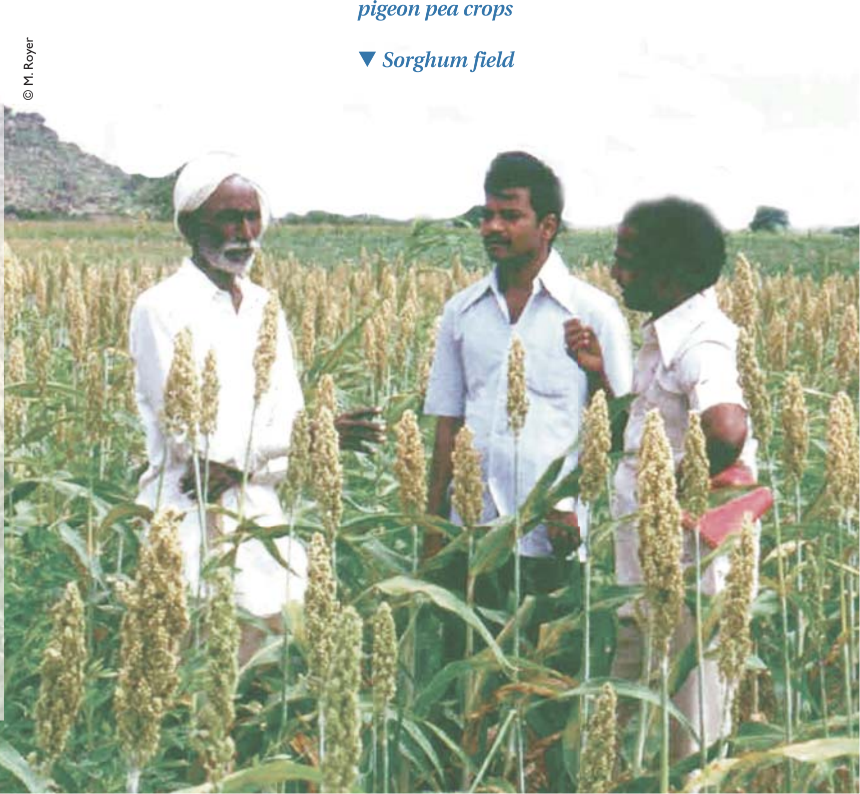
▼ *Sorghum field*

Sorghum

Sorghum is mainly used for human consumption (55%, over 600 million people depend on it) and is the 5th ranking cereal crop in the world. This staple crop is grown on an area of around 44 million ha in some 100 countries. USA, India, Nigeria and Mexico are the main producing countries.

It is also an essential food crop for millions of poor farmers in semiarid tropical regions of Africa (grown on about 14% of the arable land) and Asia since it requires less water than other cereals. Sorghum has become even more important in recent decades as it is also being cropped for forage (harvested green before seed ripening, or dry).

The area under sorghum in India is 9.2 million ha, including 5 million in the rainy season and 4.2 million ha in the dry season. Sorghum is infested by over 150 insect species, including the *Chilo partellus* stem borer, which causes reductions in both seed yield and forage quality.



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In vitro culture of pigeon pea

GMOs to the rescue of agriculture in the South?

Two categories of genetically modified organisms (GMOs) are currently cultivated—those in which a gene has been inserted which provides the plant with resistance to a herbicide, and those in which one or several genes providing resistance to some insects have been inserted (some even bear both gene types). In both cases, these GMOs are of no interest for consumers.

The first type of GMOs is produced by large multinational corporations so as to enable them to sell high volumes of herbicides. Poor farmers in developing countries manually weed their fields and the elimination of these practices would not be socially or economically interesting or feasible for the concerned governments.

It is a different story for GMOs that are resistant to pest insects. Countries that already cultivate transgenic cotton in Africa, Latin America and Asia, for instance, have noted a very sharp reduction in the quantities of pesticides used by smallholders, often with minimal precautions, and thus with substantial economic and phytosanitary gains for these users. However, here again, multinational corporations that produce the resistant seeds are the greatest beneficiaries. From a consumer standpoint, transgenic cotton does not have a softer texture nor is it cheaper than nontransgenic cotton.

The latest report of the International Service for the Acquisition of Agri-biotech Applications (ISAAA, www.isaaa.org) indicates that GMOs currently only concern two traits and four plants (soybean, maize, cotton and oilseed rape). This report also stresses that almost 80% of GMOs are only grown in five countries, i.e. USA, Argentina, Canada, Brazil and China. It is thus clear that GMOs produced so far hardly benefit poor farmers of the South.

GMO opponents as well as pro-GMO campaign instigators (mainly campaigns of governments, that especially need to market their surplus agricultural produce, to sway the opinion in favour of the benefits of GMOs in fighting hunger and poverty), therefore have solid grounds for criticizing the current debates.

But what should we think about current research that is geared towards producing GMO varieties, including research:
- carried out only by public research organizations, thus producing international public goods?
- that uses open source technologies and genes?
- involving only transfers of genes between plants (not from a microorganism or animal to plants) and often between very closely related plants?
- concerning plants that provide staple foods for the poorest people?
- on traits that are the main constraints to agricultural production in marginal agroecological areas where the poorest farmers are concentrated?
- on nutritional traits, thus aimed at improving the health of the most disadvantaged people?
- that is only carried out when conventional breeding strategies have failed, or are impossible (e.g. because of a particular reproduction mode of the plant), or very difficult due to the nature or origin of the sought-after trait?
- that has successfully passed all the biosafety tests (for the environment and health) according to current international standards?

Many public research teams (but unfortunately very few in France) are presently working along these lines, with the aim of developing future GMOs that could benefit farmers and/or consumers who are most in need of innovations.

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Note: the opinion put forward here is only that of the author and does not in any way represent an institutional position.

Conclusion and prospects

The cooperation between France and ICRISAT is mutually beneficial. French institutions have benefited by gaining access to the ICRISAT *in vitro* culture laboratory and have also been able to test their research strategies on original plants. Indian researchers at ICRISAT (and, indirectly, the international community that they serve) now have a supply of transgenic sorghum and pigeon pea plants that were planted in the field in 2003. They can thus begin a series of evaluations (including biosafety tests) and transfers of acquired resistances into different varieties—these initiatives will take several years to complete.

ICRISAT[1] scientists hope to ultimately obtain insect-resistant sorghum and pigeon pea varieties that can be cropped by farmers.

This ultimate aim could be hard to fulfil because of: (i) the many legally binding toxicological tests required prior to marketing new transgenic plant varieties, and (ii) the many patents held by multinational corporations concerning the use of *B. thuringiensis* toxins and transgenesis techniques.

1 Unfortunately, no prospects for further collaborations between CIRAD and ICRISAT are presented here since the French team concerned is no longer conducting research on this topic. Future prospects therefore only concern studies undertaken by ICRISAT.

▷▷ Publications

Sorghum project

Girijashankar V., Sharma H.C., Sharma K.K., Swathisree V., Prasad L.S., Bhat B.V., Royer M., San Secundo B., Narasu M.L., Altosaar I., and Seetharama N., 2005. Development of Transgenic Sorghum for Insect Resistance against the Spotted Stem Borer *(Chilo partellus)*. Plant cell reports 24: 513-522.

Pigeon pea project

Lavanya M., Uraichen S., Pieretti I., Vassal J.-M., Sharma K.K. and Royer M., 2003. Genetic transformation of pigeonpea to evaluate insecticidal genes for resistance to the legume pod borer, *Helicoverpa armigera*. Poster presented at the 7th International Congress of Plant Molecular Biology (Barcelona, 23-28 June 2003).