GENOMIC CHANGES IN TRANSGENIC RICE (Oryza sativa L.) AND POPLAR (Populus spp.) PLANTS

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Introduction

Ideally, transgenic plants should harbour a selected foreign gene in an otherwise unaltered genome. The integration of transgenes in the plant genome has been adequately studied (1, 2). On the other side, the frequent occurrence of changes in phenotypic traits that have been recorded in transgenic plants has been given little attention. For instance, changes in phenotypic traits, such as plant morphology, leaf shape and fertility, have been described in transgenic rice (3) and poplar (4). Since all presently available transformation procedures depend on a more or less prolonged period of time of *in vitro* cell growth , and since it is known that growth in the dedifferentiated state may induce genomic changes at high rate, it is possible that the genome of transgenic plants may undergo changes. This poses the following questions: are the observed phenotypic changes associated with genomic changes? are the latter transmitted to the progeny? Are changes caused by the integration of the foreign gene or by the *in vitro* culture steps required by the presently available transformation protocols?

In the present work we analyse, with molecular tools, the genome of different rice and poplar plants and show that most of them have undergone extensive changes. Appropriate controls show that these changes are associated to the *in vitro* culture of the transgenic cells prior to plant differentiation, rather than to the integration of the foreign gene.

Materials and Methods

Transgenic rice plants have been produced from microspore-derived protoplasts by treatment with a plasmid carrying the hph gene that confers resistance to hygromycin (5). Two successive generation were produced by self-pollination.

Alternatively, rice scutellar tissue was transformed using electric discharge particle acceleration to introduce the Bar gene that confers resistance to the commercial herbicide BASTA (6).

Transgenic poplar were produced by co-culturing leaves with an *Agrobacterium tumefaciens* strains carrying a *B.t.*-toxin gene, that confers insecticidal activity, and were propagated by cuttings.

The genome of a representative number of these plants was analysed with different molecular approaches. For the presence and expression of the foreign gene, PCR amplification as well as Southern, Northern and Western blotting were used. Genomic changes were investigated with molecular tools, such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism) RAMP (Random Amplified Microsatellite Polymorphism) and microsatellite amplification. In the case of poplar, bioassays for the insecticidal activity were also performed.

Results

Experimental data relative to the two plants will be presented.

In the case of transgenic rice produced through protoplast culture, the analysed material was: (a) microspore-derived embryogenic rice cells grown in suspension culture, (b) transgenic plants recovered from protoplasts produced from the cultured cells and (c) the self-pollination progeny (two successive generations) of the transgenic plants. DNA purified from samples of these materials was PCR-amplified with different random oligonucleotide primers and the amplification products were analysed by agarose gel electrophoresis. Band polymorphism was scored and used in band-sharing analyses to produce a similarity matrix. Relationships among the analysed genomes were expressed in a dendrogram. The extensive DNA changes evidenced in cultured cells demonstrated the occurrence of somaclonal variation in the material used to produce protoplasts for gene transfer. Quantitatively reduced DNA changes were also found in the resulting transgenic plants and in their self-pollination progenies.

In independent experiments, in which rice was transformed by electric discharge particle acceleration, and where the *in vitro* culture step was limited or nil, regenerated plants had no detectable genomic changes, as shown by an extensive RAPD, AFLP and RAMP analysis.

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In the case of poplar insect-resistant plants have been produced by infecting leaves with *Agrobacterium tumefaciens* carrying a binary vector containing different truncated forms of a *Bacillus thuringiensis* (*B.t.*) toxin gene under a duplicated CaMV 35S promoter. Putative transgenic plants were propagated by cuttings at two experimental farms (in Beijing and Xinjiang, China). At the 2nd-3rd year after transformation, 17 clones were selected, on the bases of insect-tolerance and good silvicultural traits, and evaluated, for insect resistance, for the presence of *B.t.*-toxin DNA fragment (Southern blots and PCR) and for the expression of the transgene (Western and Northern blots). Somaclonal variation, as suggested by the appearance of permanent changes in the shape of the leaves, was also investigated with molecular tools (RFLP, RAPD and microsatellite DNA). Bioassays with *Apochemia cineraius* and *Lymantria dispar* on the leaves of the selected clones, showed different and, in some cases, high levels of insecticidal activity. The molecular analysis demonstrated integration and expression of the foreign gene. Somatic changes were correlated to extensive genomic changes and were quantified in dendrograms, in terms of genomic similarity.

Conclusions

While confirming previous evidence on the stability of the foreign gene in transgenic plants and in their sexually or clonally propagated progeny, this work gives molecular evidence for the occurrence of stable genomic changes in transgenic plants and points to the *in vitro* cell culture as the causative agent.