Avraham Levy

Naomi Ragolski
Sharon Ayal
Cathy Bessudo
Eyal Emmanuel
Dan Frumkin
Khalil Kashkush
Shai Levyatov
Roy Opperman
Hezi Shaked
Elizabeth Yehuda

The dynamic plant genome

Department of Plant Sciences

Tel. 972 8 934 2734 **Fax.** 972 8 934 4181
E-mail: avi.levy@weizmann.ac.il

We are studying the mechanisms that are responsible for the rapid evolution of plant genomes. This includes homologous and non-homologous recombination and mutagenesis via transposable elements, point mutations, insertions and deletions. In addition, we are using these mechanisms to develop new tools for functional genomics in plants. In the past, we have focussed on the type of rearrangements catalyzed by these mechanisms as well as on the mechanistic aspects of DNA repair. In recent years our work has shifted to identifying the proteins that perform and regulate DNA repair and thus control plant genome stability. In addition, in collaboration with Prof. Moshe Feldman, we studied various aspects of wheat evolution.

DNA recombination mutants and genes

We have isolated the first hyper-recombinogenic mutant in plants (Hyrec). We are studying the molecular basis of the Hyrec phenotype and we are testing Hyrec for enhanced rates of gene targeting. We have also isolated new mutants which, like Hyrec, are resistant to gamma irradiation. These mutants are being tested for enhanced homologous recombination (HR) rates. In addition, we are using a reverse genetics approach, namely we identify target genes based their similarity to known genes (see below) and we screen mutant populations for mutations in these genes. For example, DNA helicases control processes such as DNA unwinding, branch migration and chromatin remodelling. Based on data from yeast, showing that the RAD54 (ATP-dependent DNA helicase) gene is affected in gene targeting, we have chosen the RAD54-like gene family in plants as a target for knockout and over-expression studies. Approximately 30 genes were identified in the fully sequenced Arabidopsis genome. We are screening for mutants in those genes, and we are in the process of silencing these genes via RNAi. In addition the yeast RAD54 gene and the E. coli RecG gene (also a DNA helicase) will be overexpressed in plants. Both mutants and over-expressers will be tested for enhanced HR and gene targeting. Another series of targets we work on are the DNA mismatch repair genes which when mutated were shown in other species to be affected in HR. Some of the mismatch repair mutants are Hyper-recombinogenic. We are using the same approach as described above for RAD54 (knockout, RNAi and overexpression) to analyze the ~15 members of this gene family in Arabidopsis. These mutants may lead both to understand the control of genome stability and to develop gene targeting in plants.

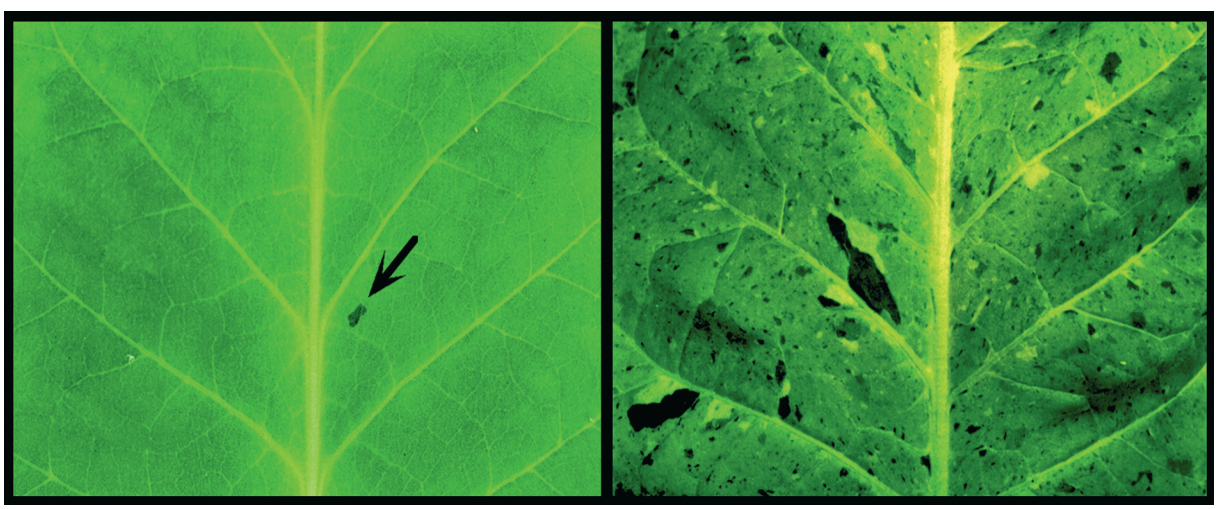

Fig. 1. *The hyperrecombinogenic mutant Hyrec (right) is characterized by the large amount of sectors compared to wild type (left).*

Forward and reverse genetics in tomato

We have developed a new tomato model system, based on Micro-tom, a miniaturized cultivar, transformed with the maize Ac/Ds elements. This system enables high-throughput functional analysis of the tomato genome. A series of new tomato genes were discovered using the Ac/Ds-Micro-Tom system, among those, a new phosphate transporter that may enable to engineer plants more efficient in Pi uptake, genes controlling flower development, genes controlling wax biosynthesis in the cuticule of various plant organs, etc. We have optimized a system whereby the flanking sequences of a transposon can be efficiently isolated and spotted on a filter or microarray for both expression studies and for screening for insertions into target genes. In addition, we have prepared fast-neutron and EMS-mutated Micro-Tom populations. The Fast Neutron population has already been useful for identifying and isolating new genes in tomato (e.g. genes affecting the beneficial colonization of the roots by Mycorrhyzae and a gene affecting carotenoid biosynthesis in tomato). The EMS population is being used for reverse genetics applications using new methods for point mutations detection. Tomato mutants will be used to characterize the genes that control homologous recombination in this species.

Wheat evolution

The wheat group contains diploid, tetraploid (e.g. durum wheat used for pasta) and hexaploid (e.g. bread wheat) species. It offers a good model to study the effect of polyploidy on genome evolution. We have analyzed, in collaboration with Prof. Moshe Feldman, the genetic and epigenetic changes that occur in the early stages of polyploid formation. We showed that DNA loss and Cytosine methylation, of both coding and non-coding regions, are the rapid response of the genome to allopolyploidy. Gene loss and gene silencing contribute to the rapid diploidization of the wheat genome. We are studying the underlying mechanism(s) of amphiploidy-associated gene loss. Another aspect of wheat evolution studied in collaboration with Prof. Moshe Feldman and Prof. Steve Weiner is evolution under domestication. We have taken a genomics approach to identify the genetic changes that occurred throughout domestication. Among the candidate genes we are analyzing, we will focus on genes that control dispersal of the grains (the Fragility genes and the free threshing genes). Mutations in these genes were selected by Human to facilitate wheat harvest. These genes will be analyzed in modern and wild wheat and in ancient wheat from archeological samples. This analysis should provide insight as to where and when wheat was domesticated.

Selected Publications

Gorbunova, V. and Levy A.A., (2000) Analysis of extrachromosomal Ac/Ds transposable element. Genetics: 155, 349-359.

Meissner, R., V. Chague, Q. Zhu, Y. Elkind, E. Emmanuel, and Levy A.A., (2000) A high throughput system for transposon tagging and promoter trapping in tomato. Plant, 22, 265-274.

Gorbunova, V., Ramos, C., Hohn, B., and Levy A.A. (2000) A nuclear protein that binds specifically to several maize transposons is not essential for Ds1 excision. Mol. Gen. Genet. 263, 492-497.

Gorbunova, B., Avivi-Ragolski, N., Shalev, G., Kovalchuk, I., Abbo, S., Hohn B and Levy, A.A. (2000) A new hyperrecombinogenic mutant of Nicotiana tabacum. Plant 24, 601-611.

Rubin, E., Lithwick, G., and Levy A.A. (2001) Structure and evolution of the hAT transposon superfamily. Genetics 158, 949-957.

Shaked, H., Kashkush, K., Ozkan, H., Feldman, M., and Levy A. A. (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell, 13, 1749-1759.

Ozkan, H., Levy A. A. and Feldman, M. (2001) Alloploidy-induced rapid genome evolution in the wheat (Aegilops-Triticum) group. Plant Cell, 13, 1735-1747.

Hohn, B., Levy, A.A. and Puchta, H. (2001) Elimination of selection markers from transgenic plants. Curr. Opini. Biotechnol. 12, 139-143.

David-Schwartz, R., Badani, H., Wininger, S., Levy, A.A., Galili, G. and Kapulnik Y. (2001) Identification of a novel genetically controlled step in mycorrhizal colonization: plant resistance to infection by fungal spores but not extra-radical hyphae. Plant, 27, 561-569.

Acknowledgements

This work was supported by the Israeli Ministry of Science, BARD, BSF, and Minerva.