Aim of the project: INRA-KSU crop biotechnology project aims to promote technological advances to manipulate endogenous plant genes without producing GMOs using in the first instance the tools developed by INRA. In the first phase of this project we aim to select a list of crops relevant to the two countries on which INRA TILLING technology will be applied. We will select crop agronomic traits that are strategic for the agriculture of the two countries and because they have impact on functional properties of the harvested crop for use in food and industrial products. This Project will also provide a solid ground to promote collaboration between the two institutions. The agreement was signed on 27 March 2008 in Paris by His Excellency Prof. Abdullah A. Alothman, Rector of King Saud University and Dr. Bernard CHARPENTIER, Director of International Relations INRA. The total budget is 1.132.000 Euro for four years period. This project is supervised by the Vice Rector of Knowledge Exchange and Technology Transfer at KSU.

Scientific context: TILLING (Targeting Induced Local Lesions IN Genomes; Colbet et al, 2001), offers an alternative way to manipulate endogenous genes for the improvement of crops without transgenics. In the basic TILLING method, seeds are mutagenized by treatment with Ethyl-Methane Sulfonate (EMS). The resulting M1 plants are self-fertilized, and the M2 generation of individuals is used to prepare DNA samples for mutational screening while their seeds are inventoried. DNA samples are pooled, and pools are subjected to gene-specific PCR. The amplification products are incubated with an endonuclease, ENDO1, that preferentially cleaves mismatches in heteroduplexe DNA (Triques, 2007). Upon detection of a mutation in a pool, the individual DNA samples are similarly screened to identify the plant carrying the mutation. This target gene modification system has many merits. First, it can be automated in a HTP mode, which is an absolute necessity to match the speed at which candidate genes are discovered (Dalmais et al 2008). Second, it is an efficient way to isolate allelic series in a specific gene and consequently identify alleles with a higher potential agronomic value. Third, it is very advantageous for searching mutants in redundant genes, which is extremely difficult using normal phenotype-based screening, like in classical breeding. Gene’s redundancy is a factor that must be taken into account, especially in crops most often carrying numerous sequences duplications and polyploid genomes.

Research areas: Four distinct research areas are at the core of our project: (1) The production and management of large collections of chemically mutagenised populations, (2) The development of HTP tools for rapid and systematic identification of mutations in target sequences, (3) Proof of concept, a list of target genes of agronomic importance to be TILLED in crops and (4) The creation of interactive and evolving databases.

Deliverables:
D1. Establish crop TILLING platform in KSU and INRA-URGV.
D2. Identification of alleles of agronomic importance
D3. New varieties with improved agronomic traits
D4. Transfer tools to KSU through exchange of scientists. KSU will then develop the
TILLING on other crops relevant to the country.

D5. Training activities for PhD and M. Sc students, scientists, and technicians.
D6. Organizing workshops of one week that will focus on practical aspects of the research tasks addressed in the project.

**Plant breeding and biotechnology at KSU:** The Unit of Breeding and Improvement of Vegetable Crops (UBIVC) was established in 2005 at the Department of Plant Production, College of Food and Agricultural Science at King Saud University. Presently, UBIVC focuses on economically important vegetable crops in Saudi Arabia such as tomato and cucurbit crops. Research has started in this unit aiming at producing genotypes that can tolerate the adverse environmental conditions (high temperature, drought and salinity). We cooperate with international research centers such as Institut National de la Recherché Agronomique (INRA) and the Asian Vegetable Research and Development Center (AVRDC) in Taiwan. The UBIVC fully cooperate with relevant research centers such as Center of Excellence in Biotechnology Research (CEBR) and Unit of Plant Biotechnology (UPB). The PBU has many modern equipments required in general biotechnology works. Examples include: PCR machines, Freeze Drying, 3130 XL DNA Sequencer and Genotypers, Real time PCR LightCycler 1.5 and Particle Gun with accessories.

Among the Research Programs being conducted in Tomato Breeding and Improvement Program are:

2. Evaluation and Improvement of Some Tomato Cultivars and Production of New Hybrids under Different Environmental Conditions. Awarding Institute: Research Center, College of Food and Agricultural Sciences.

The available projects data were published (Alsadon et al., 2006), (Alsadon and Wahballah, 2007), (Alsadon, et al., 2007) and (Mohammed et al., 2007).

**URGV-INRA:** The major goal of my research team is to develop and apply modern genomics approaches to crop plants. The most significant achievements of the last three years were the development of HTP positional cloning and reverse genetics platforms in crops. In positional cloning of genes of agronomic importance our main focus was in melon. This far we cloned an aphid resistance gene, Vat, a powdery mildew resistance gene, Pm-W, a carmovirus resistance gene, nsv, and two sex determination genes, A and G genes. In TILLING we focused our effort on the main limitation of the system, the diagnostic method for mismatches. A break through in TILLING and mutation diagnostic in general was achieved by research team. A new molecule named ENDO1 was discovered (patent deposited). ENDO1 was compared to CEL I in detecting different type of mutations and the superiority of ENDO1 was demonstrated. ENDO1 will be made available for this project. URGV will contribute in this project through the exploitation of its expertise in TILLING.

**Project Research Team**
**From KSU**
1- Prof. Abdullah A. Alsadon (Principal Investigator, Head of UBIVC and head of research team)
2- Prof. Abdullah A. Aldoss (Co-investigator and head of Center of Excellence in Biotechnology Research).
3- Dr. Mahmoud A. Wahb-Allah (Researcher)

From INRA
1- Prof. Abdelhafid Bendahmane (co-investigator and head of Plant Genomics Research Unit (URGV).
2- Christelle Troade (Researcher)
3- Adnane Boualem (Researcher)

Bilateral visits
Dr. Bendahmane to Riyadh on 10-11 June 2008
Dr. Alsadon and Dr. Aldoss to Paris on 25-30 January 2009

Signing of the project by His Excellency Prof. Abdullah A. Alothman, Rector of KSU and Dr. Bernard CHARPENTIER, Director of International Relations INRA.
KSU Research team with Dr. Bernard CHARPENTIER