PLNT2530
2024
Unit 10e

Applications of Plant Biotechnology
in Agriculture

Elimination of Allergens

Dodo et al. (2008) Alleviating peanut allergy using genetic engineering: the silencing of the immunodominant allergen Ara h 2 leads to its significant reduction and a decrease in peanut allergenicity. Plant Biotech. J. 6:135-145

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Genetic Engineering Strategy to Eliminate Peanut Allergy

- Background
  - Peanut a legume rich in protein, polyunsaturated fatty acids, and a good source of many vitamins and minerals

    - Most common cause of severe or fatal food-related anaphylaxis
    - In the USA 4.4 million people suffer from peanut and tree nut related allergies

Peanut contains 25-35% protein in the seed and these are storage proteins

Storage proteins occur as families of proteins and there are several different storage protein families in peanut

Peanut allergens

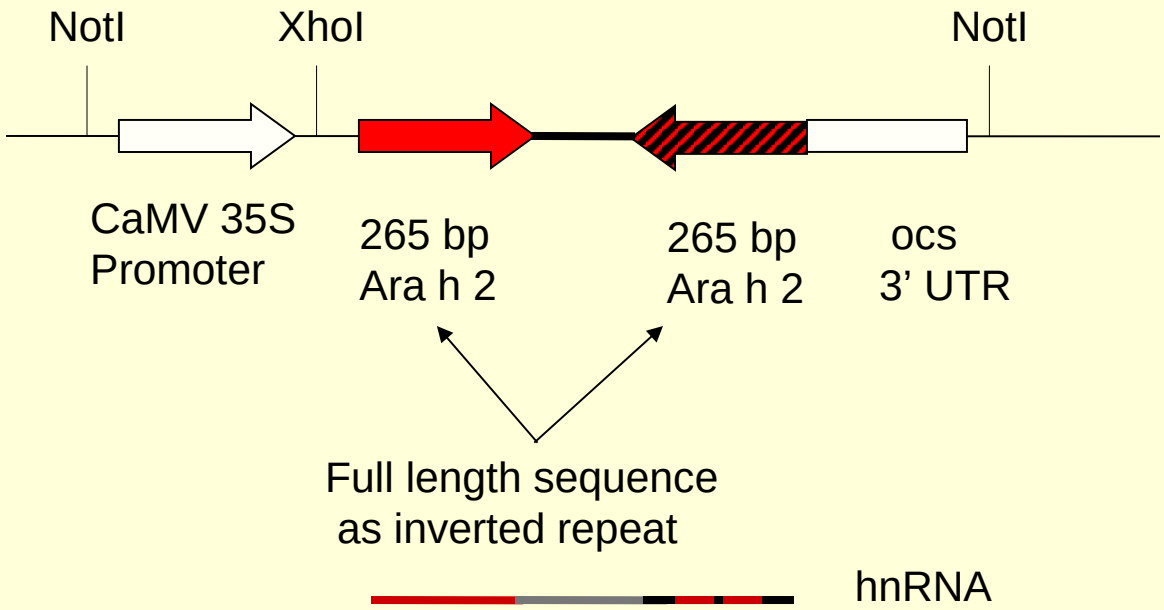
- The allergens are storage proteins and represent ~5% of the total storage protein

- 6 proteins have been identified and the most significant 3 characterized
  - Ara h 1 major allergen, glycoprotein 63.5kd, 2 isoforms
  - Ara h 2 major allergen, " 17.5kd 3 isoforms
  - Ara h 3 minor allergen, globulin 60 kd 2 isoforms

Mutation breeding would work if there were a 1 gene source of the allergen.

PTGS is better where multiple isoforms exist

Gene silencing construct



Duplex RNA subject to Dicer degradation which will trigger gene silencing of all isoforms of Ara h 2

Used to transform peanut

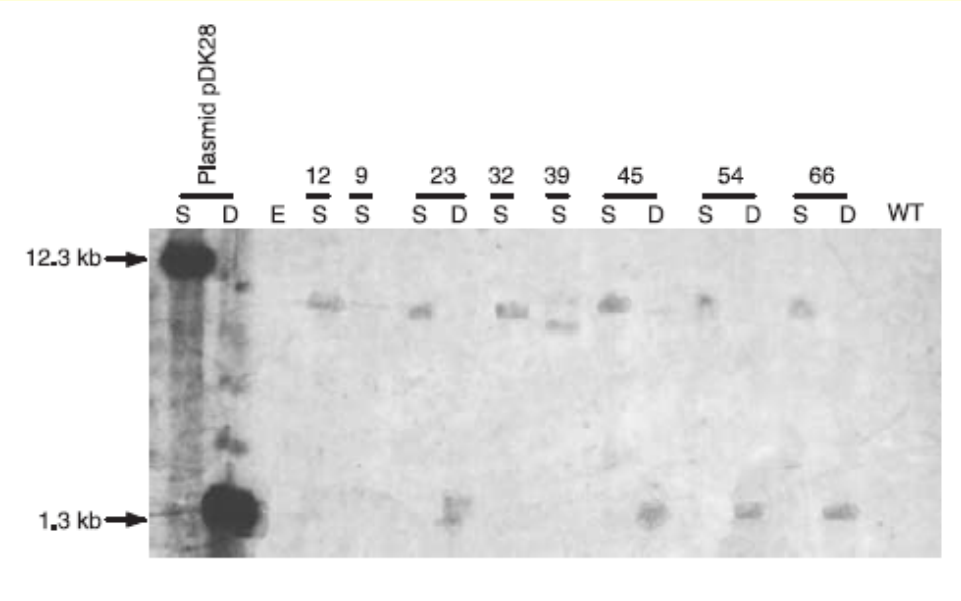
Demonstration of single copy insertions

Southern blot of T_0 peanut plants

Probe: 35S promoter fragment

WT - wild type peanut
number - transgenic progeny
S - single cut with XhoI
D - double cut with XhoI and NotI to release 35S fragment

Fragments in S lanes vary in length because they include flanking chromosomal DNA from different insertion sites.



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Demonstration of protein silencing

ELISA assay for Ara2 protein from peanut seeds.

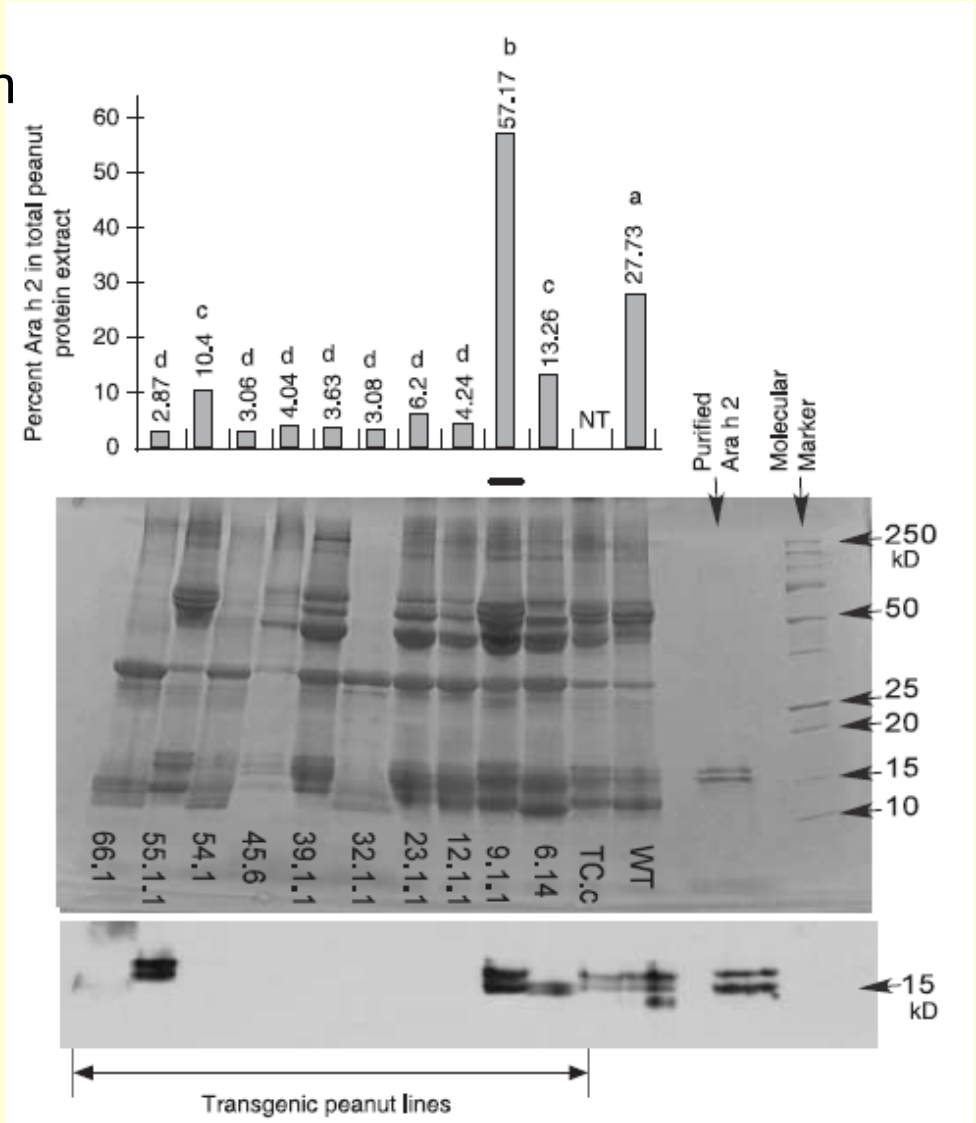
Numbers followed by same letters are not significantly different.

Coomassie blue stained SDS-PAGE gel of proteins from transgenic peanuts

WT - wildtype seeds

TC.c - WT tissue culture cells

Western blot with anti-Arah2 antibodies



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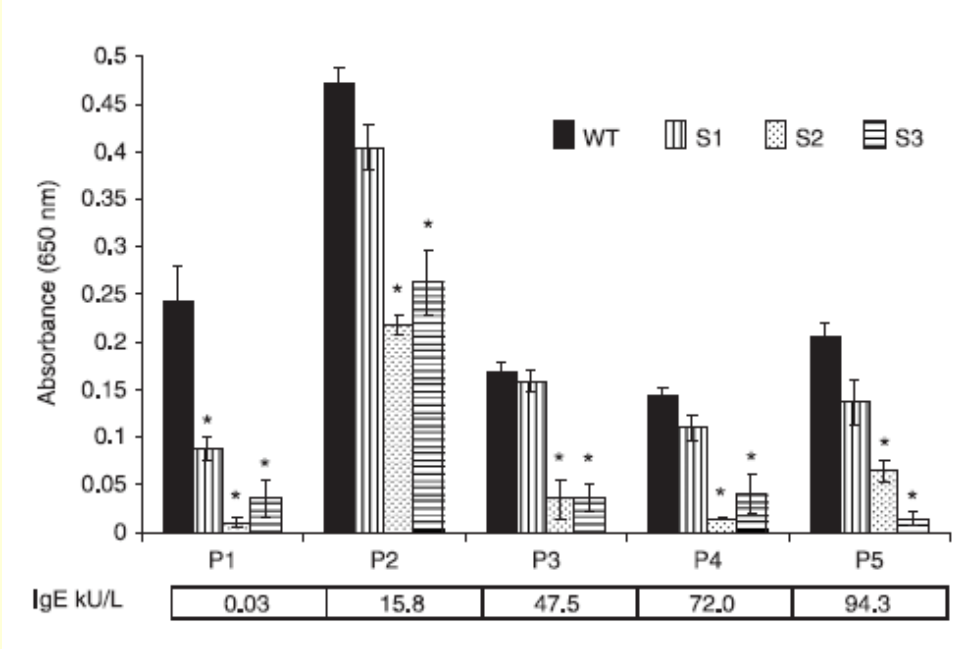
Demonstration of decreased allergenicity

IgE - immunoglobulin mainly responsible for allergic response
Antiserum for five patients with varying sensitivities to peanut were tested for IgE binding capacity with proteins from WT or gene-silenced peanut. (Remember, Arah1 and Arah3 are still active).

S1 (12.1.1), S2 (32.1),S3(45.6) - transgenic lines with no detectable Arah2 protein
WT - wild type

IgE kU/L - strength of a patient's allergic reaction, to wild type peanut (0.03 is "equivocal reaction")

A650 represents amount of Arah2 bound by IgE.



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Conclusions:

- No phenotypic differences seen between WT plants and transgenics
- Results from the 2008 paper are for T_0 ie. hemizygous plants.
- Gene silencing is usually more effective in homozygous progeny.
- Four US patents issued on this technology
- Good start, but you still need to knock out other allergenic proteins
- Would CRISPR technology be more efficient at knocking out all copies of the Arah genes?

Alternative approach:
enzymatic treatment of peanuts to degrade Ara h proteins

Dr. Jinmei Yu, North Carolina Ag & Tech. State University
Xemerge, Toronto

Method:

- shelled, skinless peanuts
- grind into a flour
- treat with food-grade enzymes that partially break down proteins

Results:
non-allergenic in clinical tests

Advantage: Simple technique using off-the-shelf food processing methods

Disadvantage: Processing must be done to every batch of peanuts;
GM crop would need no processing, and be cheaper in the long term.

http://blogs.usda.gov/2014/08/26/allergy-sufferers-may-soon-be-able-to-find-a-peanut-and-eat-it-too/

First do no harm:
Do we need to worry about allergenicity for every novel protein that we express in crop plants?

Anecdotally, we know that most allergens are proteins that are highly expressed, such as seed storage proteins. Can we assume that, for example, enzymes that tend to be transcribed at low levels, are also unlikely to be allergenic?

The AllFam database groups known allergenic proteins into families and subfamiles.

http://www.meduniwien.ac.at/allfam/browse.php

It is true that the majority of allergen familes are in fact highly expressed proteins from seeds and pollen, including storage proteins and components of the cytoskeleton (eg. tropomyosin from animals), there are some surprising allergens including lipases, chitinases the 60S acidic ribosomal protein from fungi, and heat shock protein 70 (a stress-related protein).

The answer is that we can't predict, in advance what sort of proteins might be allergenic, if we express foreign proteins in plants. For this reason, most regulatory protocols require that any novel protein be tested for allergenicity.