Genetic Improvement of Crop Plants Through Precision Breeding

Applications of PB for grape

“An advanced method for crop improvement”
Precision Breeding

A method of crop genetic improvement in which DNA sequences of known function are transferred between sexually-related species, using the mitotic pathway, to produce progeny with specific traits.

Outcomes are significantly more predictable than that from Conventional Breeding where ½ of the genes from each of two parents are uncontrollably shuffled and combined during meiosis (sexual reproduction).

http://mrec.ifas.ufl.edu/grapes/genetics
Google: “USDA GRAPEVINE 4/2/12”

PB Grapevine Approval

To: Dr. Dennis Gray, Professor
Re: APHIS confirmation of the regulatory status of grapevine with genes and regulatory elements from grapevine

Therefore, APHIS does not consider this genetically engineered grapevine as described in your February 8, 2012 letter to be regulated under 7 CFR part 340.

Please be advised that the use of these grapevines lines may still be subject to other applicable regulatory authorities such as EPA and FDA.

Google : “EPA PIP 3/16/11”

Proposed EPA Policy

The draft proposed rule will propose codifying data requirements that specifically address the registration data needs of plant-incorporated protectants (PIPs).

Also, EPA will propose to exempt cisgenic PIPs from registration to encourage research and development of useful biotechnology and reduce the number of PIPs seeking registration.

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The Basic Tenet of Crop Improvement

Without Exception

ALL crops used for food and fiber have been intentionally genetically modified by humankind. It’s a fact that every fruit, vegetable & grain, including all organics, that we purchase from the produce aisle have always been genetically modified.

Beginning in the Paleolithic era, humans began to intentionally select seeds from only the best plants in their fields. They knew that, by planting only these, next year’s yields would be better. In fact, “domestication” of plants from the wild has long been considered to be the very cornerstone of civilization.

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The Origins & Development of Crop Plants

The “Columbian Exchange” refers to the movement of plants, animals & diseases between eastern & western hemispheres due to human activities.
Some Examples of Early Crop Improvement

Tomato originated in South America. It was cultivated by the Aztecs and came to Europe as early as 1493, where it was considered to be poisonous and grown only as an ornamental. By 1692, it was being used for cooking in Italy.

There was no tomato-based Italian cuisine before then!
Some Examples of Early Crop Improvement

Corn originated as a large-seeded grass plant called “teosinte” in Northern Mexico. Over millennia, it was intentionally selected by native Americans to become the “modern” corn that was encountered by Europeans.

The modification accomplished by native Americans was orders of magnitude greater than ever could be achieved by conventional breeding.

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Most Plants and Animals have 2 sets of chromosomes composed of double-stranded DNA. Before simple cell division (Mitosis) or sexual reproduction (Meiosis) another double-stranded copy (arrow) is created in order to pass traits to offspring.

www.healthyaeon.com
The Basis of Conventional Breeding (2)

Conventional breeding utilizes the plant’s sexual reproductive cycle, in which “meiosis” functions to create gametes, each with ½ of the parental DNA. Male & female gametes then fuse to reconstitute progeny with the full, but rearranged, amount of DNA.

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Sexual Reproduction (Meiosis)

- Normal 2N cell w. two sets of chromosomes
- 1N gametes w. one chromosome set
- Division

Fertilization (Syngamy)

- 1N gametes w. one chromosome set
- Fusion
- Back to normal 2N cell

Images courtesy Luk Cox

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Meiotic Variation

1) By evolutionary design, meiosis functions to create significant genetic variation through recombination, crossing over and transposition.

2) Meiosis arose as a survival mechanism to insure adaptability to changing environments.

3) Although meiosis is necessary for breeders to generate variation, it is not optimal in instances where only specific traits are desired, but additional variation is not.

Crossing Over

Transposition

85% of the maize genome structure occurred via transposition.

Vitis contains over 1k complete copies and over 2k incomplete copies of transposons.

“Genome-wide analysis of the transposons of grapevine.”

Benjak A, Forneck A, Casacuberta JM. PloSOne 3: e3107n 2008

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The Genesis of Precision Breeding

Precision breeding is emerging only now for grapevine and many other crops due to the development and merger of three distinct technologies:

1) Manipulation of single cells to grow into plants

2) Molecular genetic tools to insert genes into plant chromosomes

3) Advancements in genome sequencing to identify genes and other elements of known function

In this way, genetic sequences of known function are incorporated into cells that develop into plants, which express the desired trait(s)

http://mrec.ifas.ufl.edu/grapes/genetics
Enabling Precision Breeding in Grapevine:
# 1 Single Cell Regeneration

These are embryogenic cultures of ‘Thompson Seedless’ that exhibit single cell regeneration

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Enabling Precision Breeding in Grapevine: # 2 Gene Insertion & Expression

Use of the green fluorescent protein (GFP) marker gene tool enables early detection of gene action in prototype optimization experiments.

Transient expression of GFP 48 hr. after gene insertion

Stable expression of GFP in all tissues via Agrobacterium mediated gene insertion

http://mrec.ifas.ufl.edu/grapes/genetics
Enabling Precision Breeding:
# 3 Genetic Analyses & Phenotype Evaluation

Examples of gene/promoter systems in field tests:

*Vitis vinifera MybA1*  -  Prompts anthocyanin production

*VvTL-1*  -  Fungal disease resistance

*VvPR-1*  -  Fungal and bacterial disease resistance

*VvAlb*  -  Fungal disease resistance

Precise Breeding of Grapevine for Improved Traits
Plant Science 228:3-10 (2014) & MREC webpage

http://mrec.ifas.ufl.edu/grapes/genetics
’Merlot’-Derived VvMyb1A Anthocyanin Transcription Factor

Controlling anthocyanin expression for several uses

1) grape-derived marker gene

2) Control of quality traits

3) Promoter analyses

Large-scale characterization of promoters from grapevine using quantitative anthocyanin and Gus assay systems

*Plant Sci. 96:132-142 2012*

http://mrec.ifas.ufl.edu/grapes
Screening for Powdery Mildew Resistance in Greenhouse

2 months after initiation

Powdery mildew on highly susceptible MybA1 ‘Thompson Seedless’

Methods of pathogen introduction

Spray cuttings with conidia solution
Severity of Powdery Mildew in Greenhouse: ‘Seyval Blanc’ Expressing the Grapevine Pathogenesis Related Protein Group 1 (PR1) Gene

Average Score of Powdery Mildew Disease Severity (0 to 5)

Plant Type (total number of cuttings)
Detached Leaf Assay to Detect PR1 Gene-Derived Resistance to Powdery Mildew

Leaf disks sprayed with conidia

Incubation in container for 20 d

Conidiophores

Intercellular hyphae detected with trypan blue

Sporulation

http://mrec.ifas.ufl.edu/grapes/genetics
Vitis vinifera TL-1 Gene Provides Black Rot Resistance in the Field

(May 10, 2008)

'http://mrec.ifas.ufl.edu/grapes/genetics'
Field Evaluation of VvPR1 Expressing ‘Seyval Blanc’ and ‘Thompson Seedless’ in 2014

http://mrec.ifas.ufl.edu/grapes/genetics
Grape Gene-Derived Fruit Rot Resistance

**Grape** VvAlb gene

- **A**
  - Non-Engineered
  - Engineered

  ‘Syrah’
  Powdery Mildew Resistance

**Grape** VvTL-1 gene

- **B**
  - Non-Engineered
  - Engineered

  ‘Thompson Seedless’
  Rot Resistance

[http://mrec.ifas.ufl.edu/grapes/genetics](http://mrec.ifas.ufl.edu/grapes/genetics)
Observations & Summary Concerning Precision Breeding

PB circumvents the variation-inducing process of meiosis by instead utilizing the mitotic developmental pathway

_Importantly, PB is akin to the selection of useful random somatic mutations from axillary buds, but differs in that it is highly controlled and predictable_

Recognition and selection of mutated branches (sports) have resulted in strains of elite grape varieties, as well as useful traits in other crops (ex. all nectarines & pink-fruited citrus)

Compared to conventional breeding, PB causes far less perturbation of the crop plant’s genome, resulting in fewer “unintended consequences”

However, as with conventional breeding, PB varieties must undergo rigorous field testing, ultimately in grower fields

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