Biotechnology and its applications for fruit and vegetable

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Pretest (please write your name and country)

1. What is biotechnology?
2. What does DNA stand for?
3. What is GMOs?
4. Which products ……….. do contain DNA

a) Cooking oil b) drinking water c) sun dried tomato and d) frozen pea

Outline

BIOTECHNOLOGY

DISCUSSION& CONCLUSION

1

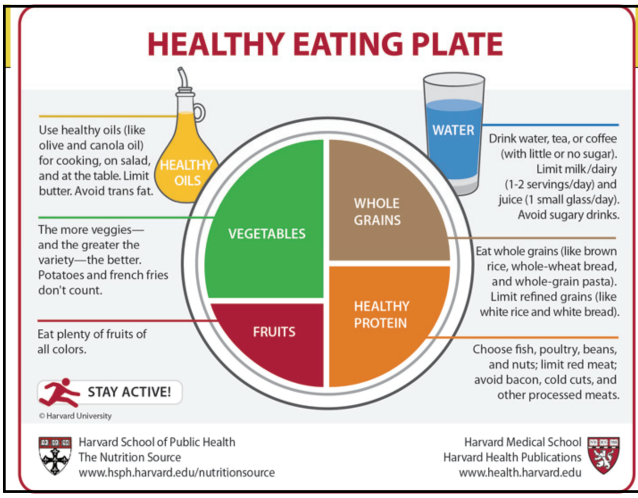
INTRODUCTION

Table 1. Comparison of horiticultural crops.

Cereals and oilseeds	Horticultural crops
Low moisture content, typically 10 to 20%.	High moisture contort, typically 70 to 95%.
Small unit size, typically less than 1 g.	Large unit size, typically 5 e to 5 kg.
Very low respiration rate with very small generation of heat. Heat production is typically 0.05 mega joule/ton/day for dry grain.	High to very high respiration rate. Heat production is typically 0.5 to 10 mega joule/ton/day at 0℃ to 5 to 70 mega joule/ton/day at 20℃.
Hard texture.	Soft texture, easily bruised.
Stable, natural shelf life is one to several years.	Perishable, natural shelf life is a few days to several months.
Losses usually caused by molds, insects and rodents sprouting and bruising.	Losses usually caused by rotting (bacteria, fungi), senescence.
Losses in LDCs usually 10 to 20%.	Losses in LDCs usually 15 to 50%

African Journal of Food Science Vol. 5 (11) pp. 603-613, 15 October, 2011

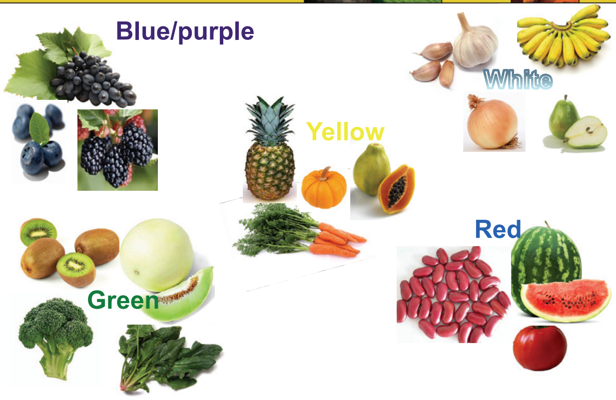
THE IMPORTANCE OF FRUITS AND VEGETABLES TO HUMAN HEALTH



5 A day-The color way

- 5 servings of fruits and vegetables a day
- 5 different colors
  - Blue/purples
  - White
  - Red
  - Green
  - Yellow

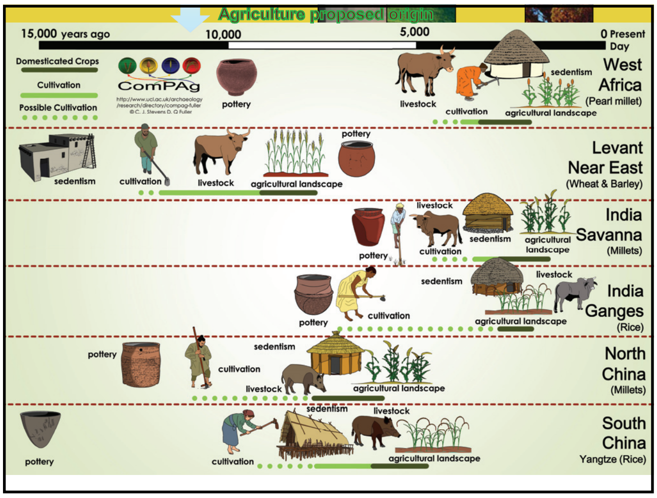
10



Food Type	1,600 Calories	2,000 Calories	Examples of One Serving
Grains At least half of your servings should be whole-grain.	6 servings per day	6-8 servings per day	• 1 slice bread • 1 oz dry cereal (check nutrition label for cup measurements of different products) • 1/2 cup cooked rice, pasta, or cereal (about the size of a baseball)
Vegetables Eat a variety of colors and types	3-4 servings per day	4-5 servings per day	• 1 cup raw leafy vegetables (about the size of a small fist) • 1/2 cup cut-up raw or cooked vegetables • 1/2 cup vegetable juice
Fruits Eat a variety of colors and types	4 servings per day	4-5 servings per day	• 1 medium fruit (about the size of a baseball) • 1/4 cup dried fruit • 1/2 cup fresh, frozen, or canned fruit • 1/2 cup fruit juice

http://www.heart.org/HEARTORG/GettingHealthy/NutritionCenter/HealthyEating/Suggested-Servings-from-Each-Food-Group_UCM_318186_Article.jsp#.ViXg_3jlmZY

HISTORY OF AGRICULTURE AND BIOTECHNOLOGY



- Eight "founder crops of agriculture"
- Emmer wheat, einkorn wheat, barley, peas, lentils, better vetch, chickpeas, flax

http://www.newworldencyclopedia.org/entry/History_of_agriculture

Flax

better vetch

Chickpea

Early on human civilization….

- Stay in one place
- More people (from hunting periods)
- Living in community and development of trade requires technologies to process extra agricultural products

Biotechnology

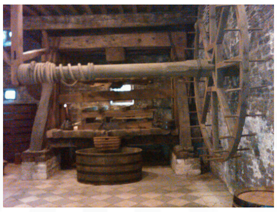
- Technology that involving living organisms (biomolecules) to transform agricultural products into new products with better taste, texture value and storage time
- Based on natural microorganisms (such as yeast) or biomolecules
- Require fermentation/incubation period
- Basic equipment/tool
- Local consumption and export

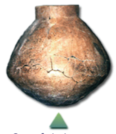
BREAD

IMAGE: Model bakery from the tomb of Meketre, chancellor to Mentuhotep II and III. From the collection of the Metropolitan Museum of Art, New York (Egypt, ~1975 B.C., plastered and painted wood, height of tallest figure is 18cm).

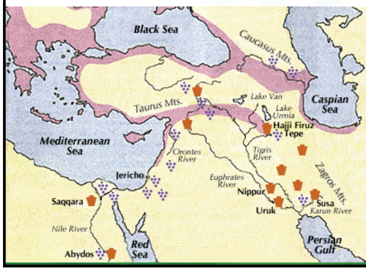
wine

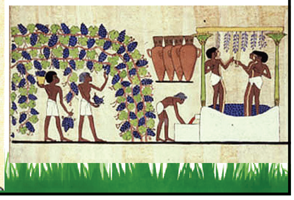
- >6,000 year old
- Found in Europe, Africa, Asia



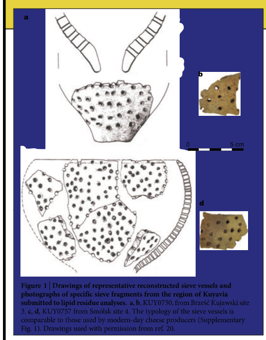


One of six jars once filled with resinated wine from the "kitchen" of a Neolithic residence at Hajji Firuz Tepe (Iran). Patches of a reddish residue cover the interior of this vessel. Height 23.5 cm. (Jar on display at the Penn Museum.)





CHEESE



- >6,000 year old
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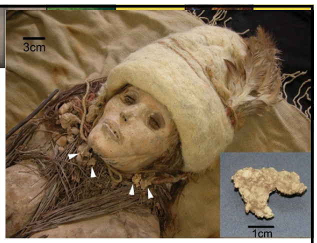
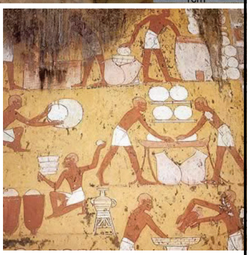
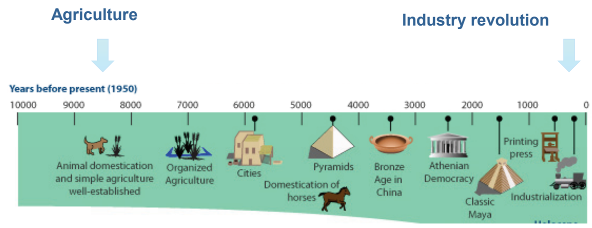
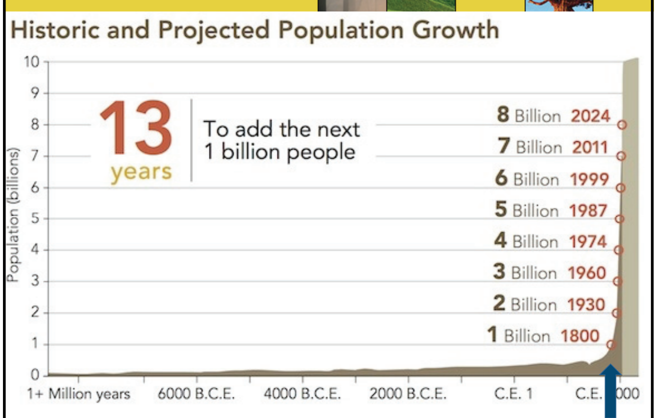






Definition of **Industrial Revolution** in English: The rapid development of **industry** that occurred in Britain in the late 18th and 19th centuries, brought about by the introduction of machinery. It was characterized by the use of steam power, the growth of factories, and the mass production of manufactured goods.



Modern ages

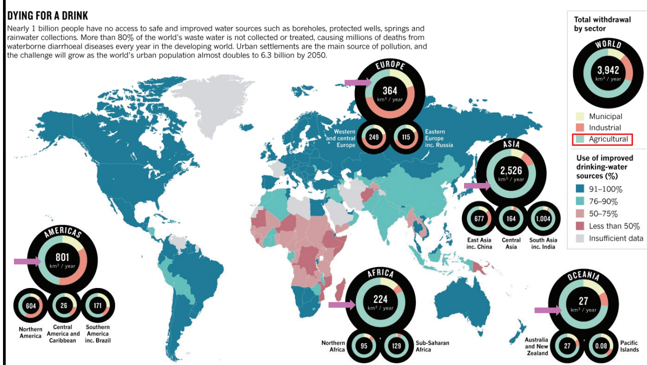
- Industrial revolution
  - Manufactured products
- More people
- Big city
- Transportation
- Life style change (single, small family, less cooking, to-go food)



Family size

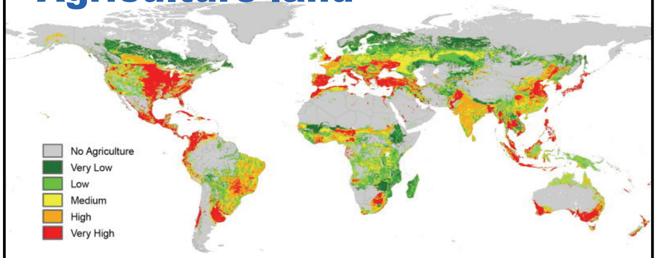

Life style

Agriculture is accounted for >70% of water usage

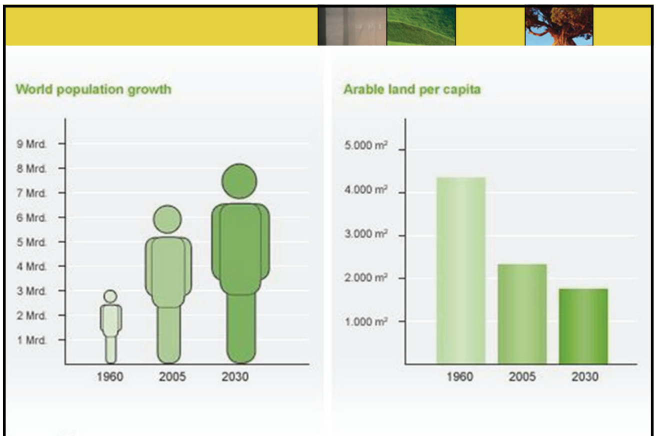


Thailand water shortage

Agriculture land

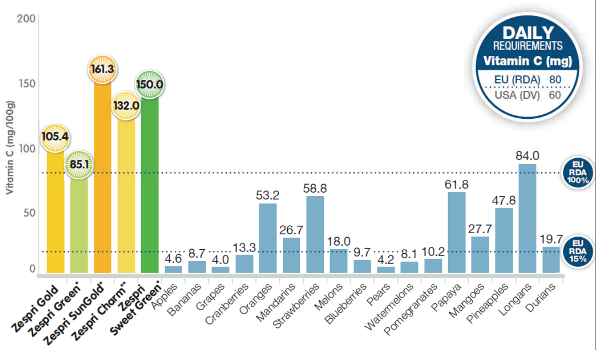


http://phys.org/news/2015-02-agricultural-insecticides-pose-global-



Source: FAO

Vitamin C content of Zespri Kiwifruit – compared with other common fruit

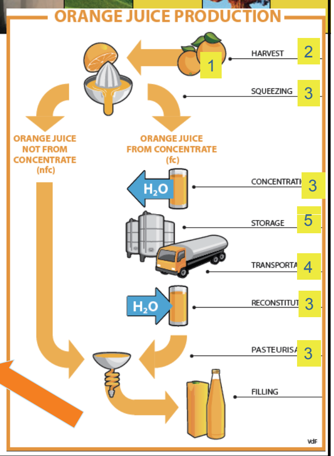


Data Sources: USDA Nutrient Database 2012 (Release 25)
* New Zealand FOODfiles 2012 Version 01
** Zespri International Limited

http://www.zespri.com/nutritious/vitamin-c

STEPS IN FRUIT AND VEGETABLE HANDLING

① Fruit and vegetable products
② Harvest
③ Processing Post-harvest
④ Package and transport
⑤ Storage
⑥ Consumption



Expectation

- Use less water, land and fertilizer
- Disease and insect resistance
- Large numbers of fruits per plants /lots of leaves
- High ratio of reproductive tissue to vegetative tissues
- High nutritional values
- Synchronize ripening/less senescent parts
- Good texture

Premium lines

- Genuine products
- Products with good texture, taste
- Product with high standards
- Product with acceptable price (good value)

Customer satisfaction

Expectation is higher hence more complicated technologies and/or new ideas are required

(Modern) Biotechnology

(MODERN) BIOTECHNOLOGY

Biotechnology is the use of living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use" (UN Convention on Biological Diversity, Art.

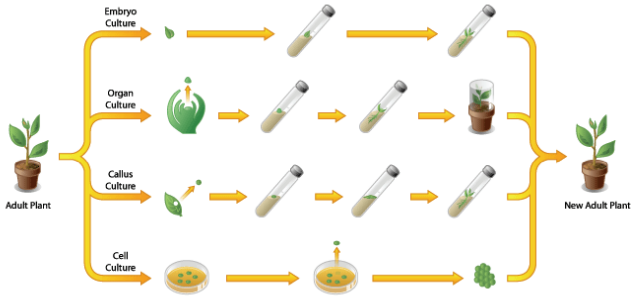
Biotechnology

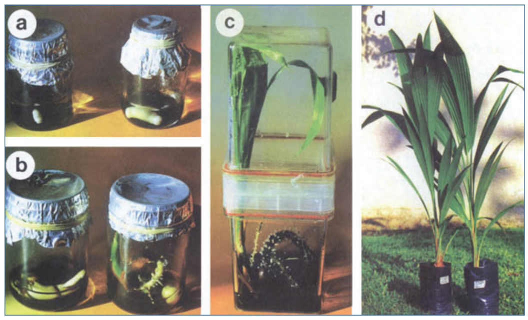
- Plant tissue culture
- Marker assisted selection
- Genetic engineering
- Disease monitoring
- Adulteration detection

PLANT TISSUE CULTURE

Plant tissue culture

- Generate plantlets/organs/un-differentiate tissues in vitro
- Synchronize production
- With/without seeds
- Using synthetic and complex media
- Sterilize condition
- Disease free



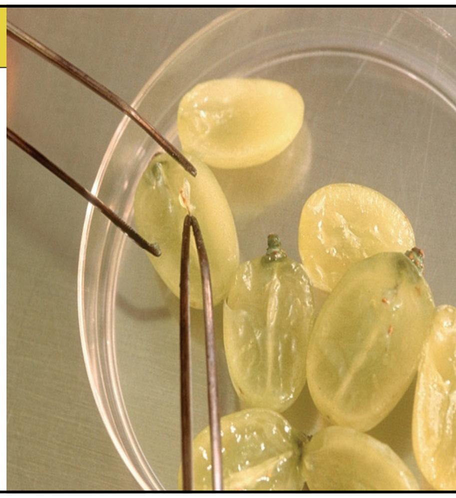


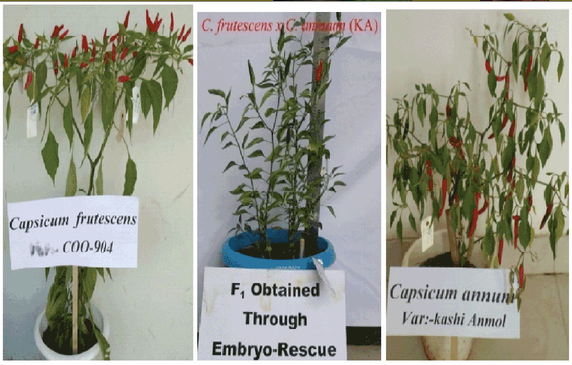
Coconut tissue culture

46

Embryo rescue

- An intro technique for saving the hybrids from fertilization
- Many embryos die at an early stage of development due to unknown reason
- Interspecific crosses between diploids and tetraploids
- Fruit crops (seedless grape, seedless lime, papaya, banana)
- Vegetables (Capsicum, onion, tomato, brinjal)
- Promote the development of weak, immature embryo into viable plants





MARKER ASSIST SELECTION

Marker assisted selection or marker aided selection (MAS)

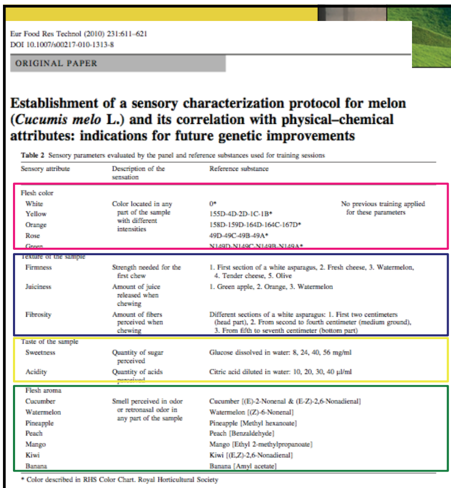
- is a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest (i.e. productivity, disease resistance, abiotic stress tolerance, and/or quality)

51

Morphological markers

- The presence or absence of morphological character such as awn, leaf sheath coloration, height, grain color, aroma of rice

52


Eur Food Res Technol (2010) 231:611–621
DOI 10.1007/s00217-010-1313-8

ORIGINAL PAPER

Establishment of a sensory characterization protocol for melon (*Cucumis melo* L.) and its correlation with physical–chemical attributes: indications for future genetic improvements

Table 2 Sensory parameters evaluated by the panel and reference substances used for training sessions

Sensory attribute	Description of the sensation	Reference substance	
Flesh color			
White	Color located in any part of the sample with different intensities	0*	No previous training applied for these parameters
Yellow		155D-4D-2D-1C-1B*	
Orange		158D-159D-164D-164C-167D*	
Rose		49D-49C-49B-49A*	
Green		N149D-N149C-N149B-N149A*	
Texture of the sample			
Firmness	Strength needed for the first chew	1. First section of a white asparagus, 2. Fresh cheese, 3. Watermelon, 4. Tender cheese, 5. Olive	
Juiciness	Amount of juice released when chewing	1. Green apple, 2. Orange, 3. Watermelon	
Fibrosity	Amount of fibers perceived when chewing	Different sections of a white asparagus: 1. First two centimeters (head part), 2. From second to fourth centimeter (medium ground), 3. From fifth to seventh centimeter (bottom part)	
Taste of the sample			
Sweetness	Quantity of sugar perceived	Glucose dissolved in water: 8, 24, 40, 56 mg/ml	
Acidity	Quantity of acids perceived	Citric acid diluted in water: 10, 20, 30, 40 µl/ml	
Flesh aroma			
Cucumber	Smell perceived in odor or retronasal odor in any part of the sample	Cucumber [(E)-2-Nonenal & (E-Z)-2,6-Nonadienal]	
Watermelon		Watermelon [(Z)-6-Nonenal]	
Pineapple		Pineapple [Methyl hexanoate]	
Peach		Peach [Benzaldehyde]	
Mango		Mango [Ethyl 2-methylpropanoate]	
Kiwi		Kiwi [(E,Z)-2,6-Nonadienal]	
Banana		Banana [Amyl acetate]	

* Color described in RHS Color Chart. Royal Horticultural Society

Flesh colour

Texture

Taste

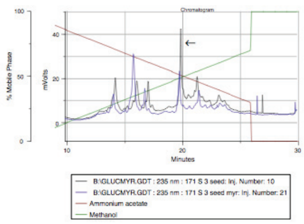
Flesh aroma

53

Biochemical markers

- Proteins or chemical produced by plants
- Enzymatic activity, HPLC

nicotianamine synthase



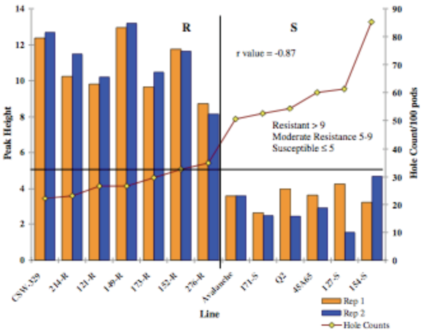


Fig. 3 Comparison between replicated standardized peak height concentrations in upper cauline leaf extracts and larval exit hole counts from the field trial in 2007. Data are organized by ascending larval exit hole counts. The *r* value at −0.87 shows a strong significance difference between R and S

Biochemical markers for cabbage seedpod weevil (Ceutorhynchus obstrictus (Marsham)) resistance in canola (Brassica napus L.)

Euphytica (2009) 170:297–308

55

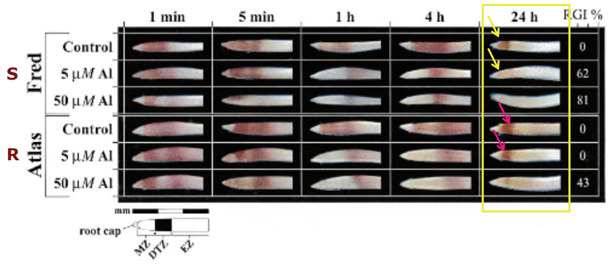


Fig. 2. Kinetics of NBT reduction activity in the Al-sensitive and the Al-tolerant cultivars exposed to various Al concentrations. Two wheat cultivars (Al sensitive, Fredrick: Fred; Al-tolerant, Atlas-66: Atlas), were exposed to the Al control solution (1 mM $CaCl_2$, pH4.15) or to the same solution containing Al with the final concentrations indicated. RGI was estimated after 24 h of Al exposure. NBT reduction was performed as in Fig. 1. The drawing represents the scale in mm and the approximate positions of the different root regions (based on Sasaki et al. 1997, and Sivaguru and Horst 1998). MZ, meristematic zone; DTZ, distal transition zone; EZ, elongation zone.

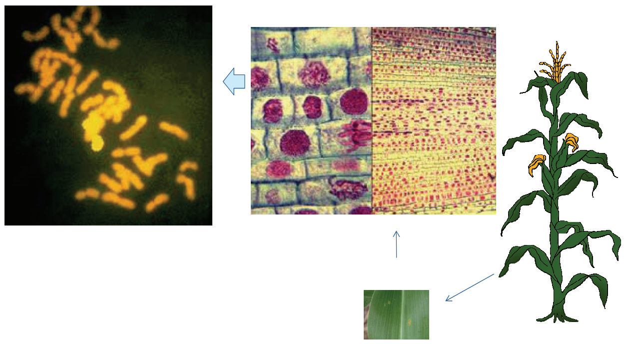
A new biochemical marker for aluminium tolerance in plants

PHYSIOLOGIA PLANTARUM 115: 81–86. 2002

56

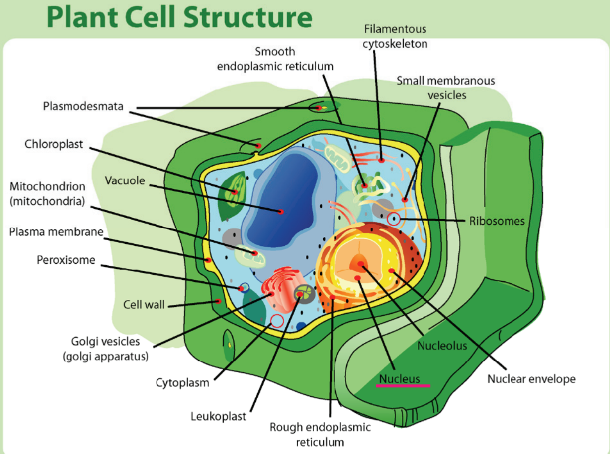
DNA MARKER

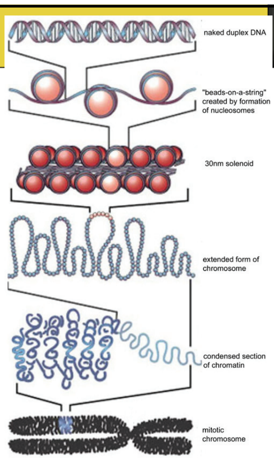
Plant chromosome

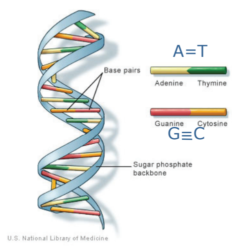


58

Plant Cell Structure







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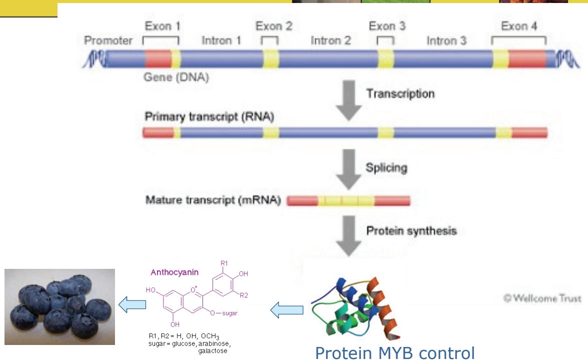


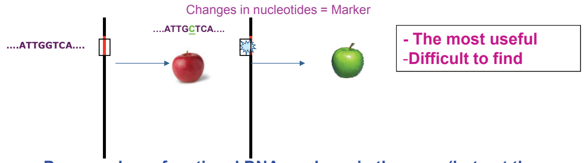
Blueberry

Protein MYB control
the production of anthocyanin

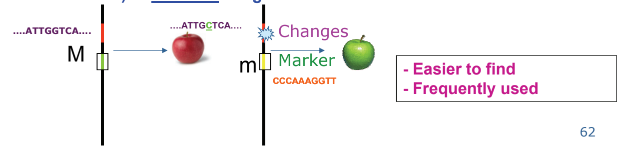
61

Two basic types of DNA-markers

- DNA marker in coding region that cause the phenotypic change



- Presumed non-functional DNA-markers, in the gene (but not the causal mutations) or linked to the gene



62

DNA marker

- The use of DNA marker to identify gene or phenotypes of plant
- DNA marker can be the target gene or closely link to the target gene
- Can be used with or without previously knowledge of target genes or relationship between DNA/gene and phenotypes

63

- it can aid the breeder in more efficiently selecting the plants or lines to move forward in the program (forward selection)
- Can also be used to improve all other trait combinations in the germplasm, either
  - by crossing out unwanted alleles or
  - maintaining those of value (background selection)

Desirable properties for a good molecular marker

* Polymorphic
* Co-dominant inheritance
* Occurs throughout the genome
* Reproducible
* Easy, fast and cheap to detect
* Selectivity neutral
* High resolution with large number of samples

Molecular markers

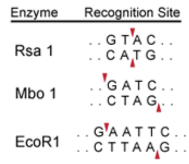
- RFLP (Restriction Fragment Length Polymorphism)
- RAPD (Random Amplified Polymorphic DNA)
- AFLP (Amplified Fragment Length Polymorphism)
- SSR (Simple Sequence Repeat)
- SCAR (Sequence Characterized Amplified Region)
- CAP (Cut/Cleaved Amplified Polymorphism)

PCR (RAPD, AFLP, SSR, SCAR, CAP)

RFLP - Restriction fragment length polymorphism

- A technique to identify a change in the genetic sequence that occurs at a site where a restriction enzyme cuts. RFLPs can be used to identify specific mutations, and as molecular markers.

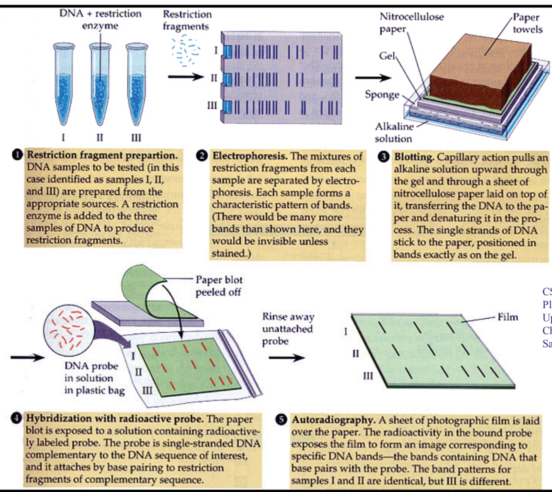
Restriction enzymes are proteins isolated from bacteria that recognize specific short sequences of DNA and cut the DNA at those sites. The normal function of these enzymes in bacteria is to protect the organism by attacking foreign DNA, such as viruses.



RsaI from *Rhodopseudomonas sphaeroides* (S. Kaplan)

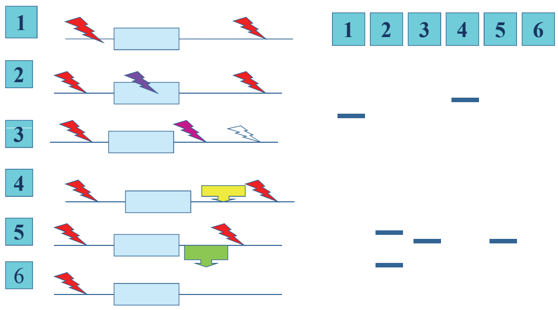
MboI from *Moraxella bovis*

EcoRI from *Escherichia coli*



CSS/HORT 430
Plant Genetics
Updated by Tanya Chutimanitsakun fr
Sagar Sathuvalli

RFLP Polymorphisms interpretation



Theor Appl Genet (2008) 118:15–27
DOI 10.1007/s00122-008-0873-5

ORIGINAL PAPER

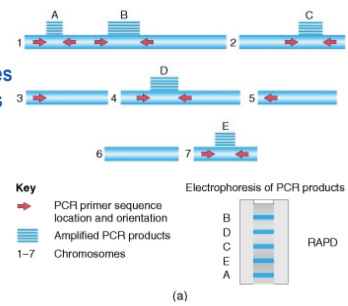
BAC-derived markers converted from RFLP linked to *Phytophthora capsici* resistance in pepper (*Capsicum annuum* L.)

Make a better marker from RFLP for pepper

71

Random Amplified Polymorphic DNA RAPD

- ✓ Amplifies anonymous stretches of DNA using arbitrary primers
- ✓ Fast and easy method for detecting polymorphisms



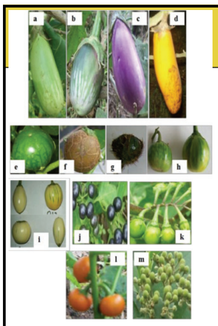


Figure 1. Variability in fruit colour and shape of some eggplant samples studied. Legend: (a - d) *S. melongena*; (e and f) *S. macrocarpon*; (g) *S. dasyphyllum*; (h and i) *S. gilo*; (j) *S. scabrum*; (k) *S. incanum*; (l) *S. aethiopicum*; (m) *S. erianthum*.

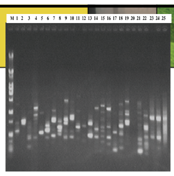


Figure 2. RAPD profiles generated by primer B-18 for *Solanum* samples studied. Legend: M represents the 100 bp DNA Ladder which serves as the reference point; 1 to 25 corresponds to bands produced by the amplified DNA from the 25 samples.

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http://www.academicjournals.org/IJGMB
International Journal of Genetics and Molecular Biology

Full Length Research Paper

Partitioning and distribution of random amplified polymorphic DNA (RAPD) variation among eggplant *Solanum* L. in Southwest Nigeria

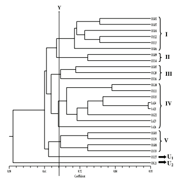


Figure 3. A UPGMA dendrogram showing genetic relationship among accessions of eggplants studied. Legend: Y represents truncated line at a co-efficient of similarity 0.65; I to V represent the five clusters that were distinguishable from the dendrogram while U_1 and U_2 represent ungrouped samples at that co-efficient of similarity.

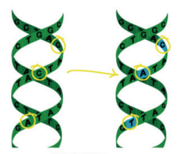
26 samples of eggplants, can be separated
by RAPD in to 5 groups

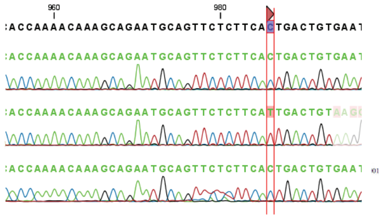
73

SNPs
(Single Nucleotide Polymorphisms)

Molecular markers which their polymorphism can be determined by single nucleotide difference

SNPs on a DNA strand





✓Any two unrelated individuals differ by one base pair every 1,000 or so, referred to as SNPs.
✓Many SNPs have no effect on cell function and therefore can be used as molecular markers.

Euphytica (2010) 171:301–311
DOI 10.1007/s10681-009-0017-2

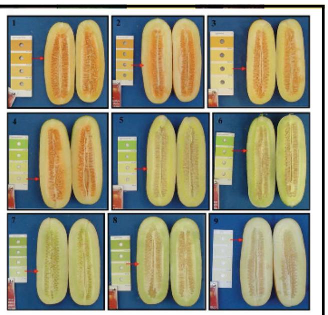
REVIEW

Inheritance of beta-carotene-associated flesh color in cucumber (*Cucumis sativus* L.) fruit

H. E. Cuevas · H. Song · J. E. Staub · P. W. Simon

Table 1 Number of samples, means and standard errors (SE) for β-carotene content (ug g^{-1}) of fruit mesocarp and endocarp classification used to characterized cucumber (*Cucumis sativus* L.) flesh color of parental lines and segregating generations (F_2, BC_1P_1, and BC_1P_2) derived from unpigmented (white) interior flesh cucumber inbred line 'Gy7' (P_1) and the pigmented (orange) interior flesh inbred line 'EOM 402-10' (P_2) as evaluated in two greenhouses in Madison, Wisc. in 2008

Color segregation[a]	Mesocarp		Endocarp	
	n^b	Means[c] ± SE	n^b	Means[c] ± SE
Orange (*ORG*)	4	2.72 ± 1.15[a]	26	7.54 ± 0.68[a]
Light orange (*LORG*)	4	1.90 ± 0.83[a]	1	3.05[a]
Yellow (*Y*)	11	0.34 ± 0.17[b]	5	0.73 ± 0.18[b]
Light yellow (*LY*)	6	0.06 ± 0.04[b]	2	0.33 ± 0.11[b]
Yellow green (*YGR*)	7	0.10 ± 0.07[b]	–	–
Green yellow (*GRY*)	5	0.07 ± 0.05[b]	2	0.19 ± 0.18[b]
Light green (*LGR*)	5	0.01 ± 0.00[b]	2	0.19 ± 0.17[b]
Green (*GR*)	5	0.01 ± 0.00[b]	3	0.37 ± 0.32[b]
White (*WH*)	42	0.02 ± 0.00[b]	5	0.16 ± 0.08[b]



An example of the use of SNP for flesh colour In cucumber

Gene symbol[a]	Cucumber EST database	NCBI	Sequence of primers used to amplify fragment used for genotyping	Annealing temp. (°C)	Fragment size (bp)	Polymorphism
PS	CU2624	GQ203104	F- CTTTGTCTGGTGATGAAGATGG R- CACGCCTTGTCAATTTGTTG	60	110	INDEL (13 bp)[c]

77

Theor Appl Genet (2014) 127:2051–2064
DOI 10.1007/s00122-014-2360-5

ORIGINAL PAPER

High-density single nucleotide polymorphism (SNP) array mapping in *Brassica oleracea*: identification of QTL associated with carotenoid variation in broccoli florets

Marker comparison

Feature	Morphological markers	Biochemical molecular markers	DNA based markers
Feature of the organism scored	Phenotype	Protein	DNA base sequence
Biological meaning of the markers	Consequences of gene action	Genes that are expressed	DNA sequences, may or may not represent genes
Plant material required for detection	Intact plant or plant organ	Little amount of tissue	Little to medium amount of tissue and no matter what tissue is used
Efforts required for detection	Simple	Moderate	Moderate to difficult
Ease of use	Very easy	Moderately difficult	Moderately difficult to difficult
Reproducibility	High	High	Moderate to high
Dominance/ Codominance	Generally dominant	Codominant	Dominant (RAPD, AFLP) Codominant (RFLP, SSR)

79

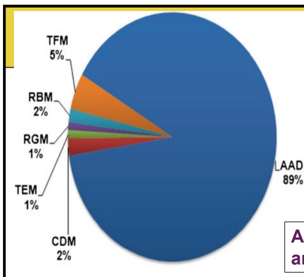


Figure 1 Percentages of studies utilizing different types of molecular markers. The chart is based on an informal literature search performed with Google Scholar on 22.08.2012 resulting in 1032570 hits. Abbreviations are according to acronyms found in the text: **AAD** – Arbitrarily amplified DNA markers, including AFLP, ISSR, RAPD, and other modified but similar methods mentioned in the text; **CDM** – conserved DNA based markers, including CDDP, PBA, TBP, ITP (all modified methods are cited in the text); **TEM** – transposable element based markers including IRAP, REMAP, ISAP, iPBS and SSAP. **RGM** – resistance-gene based markers (RGAP), NBS-profiling; **RBM** – RNA-based markers, iSNAP, EST- and cDNA- based markers; **TFM** – targeted fingerprinting markers (DALP, PAAP, SRAP, TRAP, CoRAP and SCoT).

Poczai et al. Plant Methods 2013, 9:6
http://www.plantmethods.com/content/9/1/6

PLANT METHODS

REVIEW Open Access

Advances in plant gene-targeted and functional markers: a review

Around 80% of molecular markers are arbitrary amplified DNA markers

Application of markers in crop improvement

- Selection of good characters lines from germplasm
  - Fruit characters; firmness, sweetness, colour, aroma, ethylene production
  - Morphology; shape, size
  - Abiotic stress; drought
  - Biotic stress; disease resistance
- Assist plant breeders to determine outsprings with desired characters
- Acquire lines with several characters simultaneously

81

Examples

- Chili pepper (disease resistance, pungency)
- Radish (male sterility)
- Onion (bulb colour)

Yoon et al 2015

Modification of plant genome

83

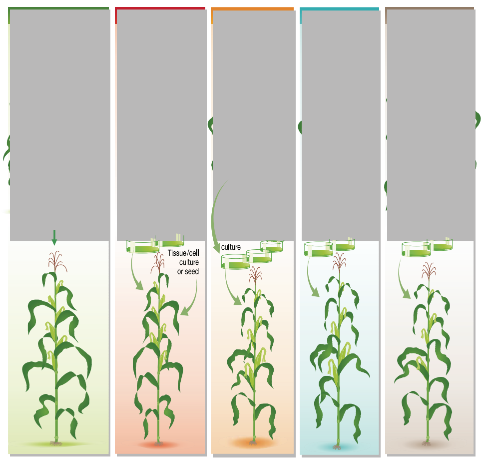
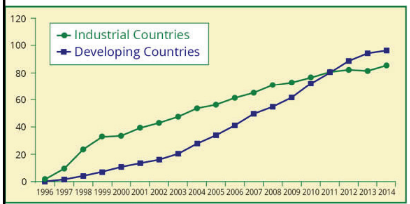


Fig 1 | Techniques that breeders use commonly to create new plant varieties. DSB, double-strand break; ZFN1/2, zinc-finger nuclease 1/2.

EMBO reports VOL 13 | NO 12 | 2012 1057

Figure 2. Global Area of Biotech Crops, 1996 to 2014: Industrial and Developing Countries (Million Hectares)



Source: Clive James, 2014.

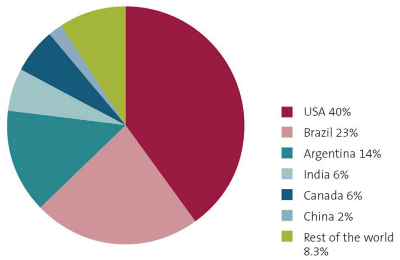
Table 1. Global Area of Biotech Crops, 1996 to 2014

	Hectares (Million)	Acres (Million)
1996	1.7	4.3
1997	11.0	27.5
1998	27.8	69.5
1999	39.9	98.6
2000	44.2	109.2
2001	52.6	130.0
2002	58.7	145.0
2003	67.7	167.2
2004	81.0	200.0
2005	90.0	222.0
2006	102.0	250.0
2007	114.3	282.0
2008	125.0	308.8
2009	134.0	335.0
2010	148.0	365.0
2011	160.0	395.0
2012	170.3	420.8
2013	175.2	433.2
2014	181.5	448.0
TOTAL	1,784.9	4,413.5

Increase of 3-4%, 6.3 million hectares (15.6 million acres) between 2013 and 2014.

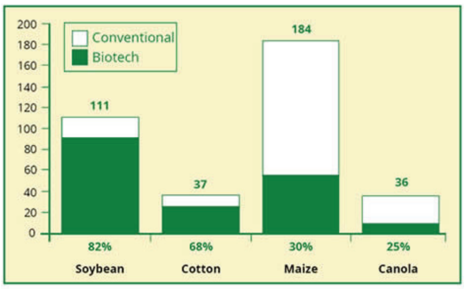
Source: Clive James, 2014.

GM PRODUCTION OF TOP SIX COUNTRIES



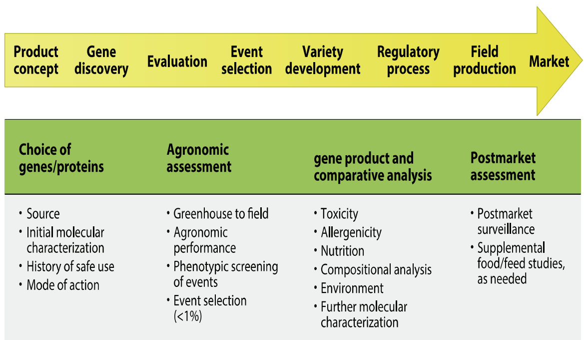
Source: *Calculations based on ISAAA (2014). Special Brief 46 – 2013 Executive Summary, Global Status of Commercialised Biotech/GM Crops: 2013, http://www.isaaa.org/resources/publications/briefs/46/executivesummary/*

Figure 3. Biotech Crop Area as % of Global Area of Principal Crops, 2014 (Million Hectares)



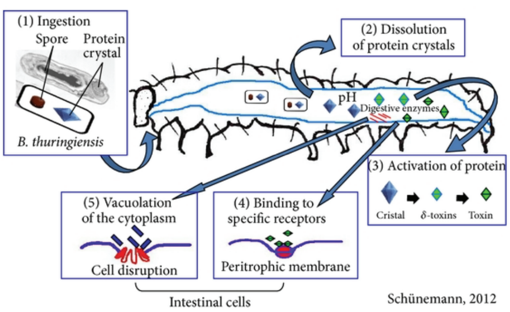
Source: Clive James, 2014.

GM plant development

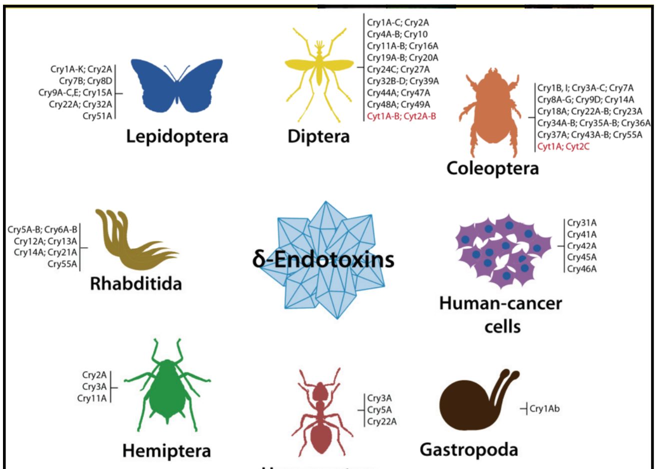


Annu. Rev. Plant Biol. 2014. 65:769–90

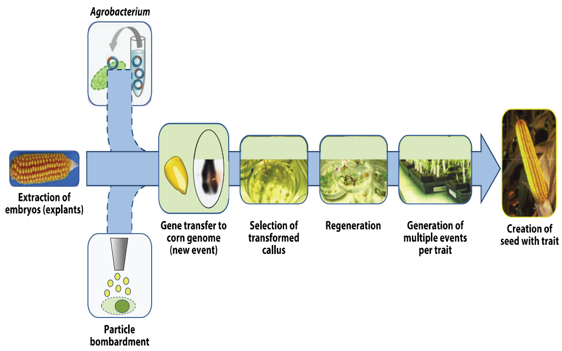
BT toxin



Schünemann, 2012



Toxins **2014**, *6*(12), 3296-3325; doi:10.3390/toxins6123296
Review: ***Bacillus thuringiensis* Toxins: An Overview of Their Biocidal Activity**



Annu. Rev. Plant Biol. 2014. 65:769–90

	Discovery Gene/trait identification	Phase 1 Proof of concept	Phase 2 Early development	Phase 3 Advanced development	Phase 4 Prelaunch
Average duration	54 months	27 months	30 months	37 months	49 months
Average cost	USD 31 million	USD 28.3 million	USD 13.6 million	USD 45.9 million	USD 17.2 million
Key activity	• High-throughput screening • Model crop testing	• Gene optimization • Crop transformation	• Trait development • Preregulatory data • Large-scale transformation	• Trait integration • Field testing • Regulatory data generation • Product development	• Regulatory submission • Seed bulk-up • Premarketing • Product development

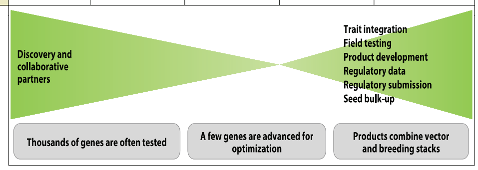


Figure 1

Overview of the development process of a genetically engineered crop, including activities, durations of those activities, and costs. Durations and costs are industry averages (60). Because various activities overlap, the cumulative total of each phase does not reflect the actual duration of the overall research and development process.

ABIOTIC STRESS RESISTANT/TOLERANT TRAITS

Table 1 Genes, mechanisms, and genetically modified fruit plant species implicated in plant responses to many abiotic stresses: some recent reports

Plant	Gene	Remarks	Perform of transgenic plants to abiotic stress	References
Apple	*Osmyb4*	Encoding a transcription factor belonging to the Myb family, accumulation of several compatible solutes	Drought and cold	Pasquali et al. (2008)
	MdNHX1	Tonoplast Na^+/H^+ antiporters	Salt	Li et al. (2010)
	PpCBF1	C-repeat binding factor (CBF/DREB), transcriptional activator genes	Cold	Wisniewski et al. (2011)
	MdCIPK6L	Encode a CBL-interacting protein kinase (CIPK)	Salt, drought and chilling	Wang et al. (2012)
Banana	*MusaDHN-1*	Overexpression of dehydrin gene, belonging to a broader class of LEA proteins	Drought and salt	Shekhawat et al. (2011a)
	MusaWRKY71	Encodes a WRKY transcription factor protein	Multiple abiotic stress	Shekhawat et al. (2011b)
	MusaSAP1	Encodes a zinc finger protein i.e. stress associated proteins (SAP)	Multiple abiotic stress	Sreedharan et al. (2012)
Citrus	*P5CSF129A*	Osmotic adjustment, protected against ROS by modulating the antioxidant enzymes activity	Water deficit	de Campos et al. (2011)
	AhBADH	Overexpressing AhBADH gene regulates accumulate higher level of glycinebetaine	Salt	Fu et al. (2011)
	D1-pyrroline-5-carboxylate synthetase (*P5CS*)	Endogenous accumulation of proline	Drought	de Carvalho et al. (2013)

Grapevine	*DREB1b*	Dehydration response element binding gene, a cold inducible transcription factor	Cold	Jin et al. (2009)
	VvCBF4	C-repeat binding factor gene, reduced freezing-induced electrolyte leakage	Cold	Tillet et al. (2012)
Kiwifruit	*AtNHX1*	Maintaining a relatively high K^+/Na^+ ratio	Salt	Tian et al. (2011)
Mulberry	*hva1*	Encodes a group 3 LEA protein	Salinity and drought	Lal et al. (2008)
	Osmotin	Encoding osmotin and osmotin-like proteins belonging to the plant PR-5 group of proteins	Salt, drought and variety of fungal (biotic) pathogen	Das et al. (2011)
Papaya	C-repeat binding factor (*CBF*)	Transcriptional activator genes	Cold	Dhekney et al. (2007)
Pear	*SAMDC2*	Encodes sadenosylmethionine decaboxylase, transgenic plants expressing polyamines	Salt	He et al. (2008)
	SPDS1, SPDS	Encodes spermidine synthase, transgenic plants expressing polyamines	Salt, multiple abiotic stress	Wen et al. (2008, 2009)
Strawberry	*Osmotin*	Enhanced levels of proline, total soluble protein	Salt	Husaini and Abdin (2008)

AGRONOMIC AND HORTICULTURAL TRAITS

Table 3 Genetic transformation of fruit plants with agronomic and horticultural traits: some recent reports

Plant	Gene	Remarks	References
Apple	Mal d 1	Development of hypoallergenic transgenic apples by inhibit the expression of Mal d 1 using RNA interference	Gilissen et al. (2005)
	BpMADS4	Shortening the juvenile phase by overexpressing the *BpMADS4* gene	Flachowsky et al. (2007)
	gai (gibberellic acid insensitive)	Reduced plant height (dwarf apple) in transgenic clones	Zhu et al. (2008)
	1-Amino-cyclopropane-carboxylase oxidase (*MdACO1*)	Anti-sense suppression of (MdACO1), low production of ethylene in fruit	Johnston et al. (2009)
	RSV-F	Encoding for the human respiratory syncytial virus (RSV)-F protein, efficacy of using apple for developing a plant-based vaccine against RSV	Lau and Korban (2010)
	rol B	Effects of *rol B* transgenic rootstocks on growth, flowering and fruit quality of non-transgenic scion cultivars grafted onto these rootstocks	Smolka et al. (2010)
	BpMADS4	Transgenic apple was characterized and selected for its use in a fast breeding program	Flachowsky et al. (2011)
	PG1	Down regulation of *PG1* expression caused fruit softening in transgenic line	Atkinson et al. (2012)
	MdTFL1-1	Reduced vegetative growth and generation time	Flachowsky et al. (2012)
	MYB10	GM apple have high concentrations of foliar, flower and fruit anthocyanins, analysis of GM apples reveals effects on consumer attributes	Espley et al. (2013)
	FLOWERING LOCUS T genes (*PtFT1* and *PtFT2*)	Improved fast track breeding approach by heat-induced expression of *PtFT1* and *PtFT2* genes	Wenzel et al. (2013)
Ci	C GA20 1 (k	A i i d d h i h f l h	F 1 (2007)

p g

Citrus	CcGA20ox1 (a key enzyme of GA biosynthesis)	Antisense expression reduced height of plant whereas sense expression promoted shoot length	Fagoaga et al. (2007)
	Arabidopsis thaliana MAC12.2 gene	Significantly less seeds in transgenic lines than the control	Tan et al. (2009)
Grapevine	rol B	Improved the rooting of grape rootstocks	Geier et al. (2008)
Kiwifruit	GGP	Increased ascorbic acid content in transgenic lines	Bulley et al. (2012)
Papaya	*ACC oxidase*	Block ethylene production and delay the ripening rate, first report of the use of cosuppression technology in a tropical fruit	Lopez-Gomez et al. (2009)
Pear	Citrus FLOWERING LOCUS T gene (*CiFT*)	Early flowering in transgenic lines	Matsuda et al. (2009)
Plum	Phytoene desaturase (*pds*)	Inhibition of carotenoid biosynthesis and chlorophyll photo-oxidation	Petri et al. (2008)
	FLOWERING LOCUS T genes (*PtFT1*)	Transgenic lines produced fruits in the greenhouse within 1–10 months	Srinivasan et al. (2012)
Strawberry	Stilbene synthase gene (*chs*)	Alterations in the phenylpropanoid metabolism	Hanhineva et al. (2009)
	rolC	Better rooting of stem cuttings and early flowering in transgenic lines	Landi et al. (2009)
	FaMYB10	Enhanced anthocyanin content in root, foliar, and fruit	Lin-Wang et al. (2010)
	FaEG3	Increased fruit firmness in transgenic line	Mercado et al. (2010)
	PFP	Over-expression of pyrophosphate: fructose 6-phosphate 1-phosphotransferase, regulates glycolytic and gluconeogenic metabolism	Basson et al. (2011)
	Endo-β-1,4-glucanase antisense gene (*FraCel1*)	Regulate starch content in fruit during ripening	Lee and Kim (2011)
	FaplC and *FaEG3* antisense genes	Encoding a pectate lyase and a endo-β-1,4-glucanase, reduced rate of fruit softening	Youssef et al. (2013)

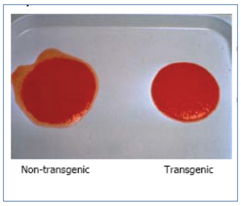
DISEASE/INSECT RESISTANT TRAITS

Table 2 Transgenic plants expressing genes for disease resistance in fruit plants: some recent reports

Plant	Gene	Resistance/disease	Remarks	References
Apple	*HcrVf2*	*Venturia inaequalis*/apple scab disease	Confers scab resistance in transgenic cultivated apple cv. Gala	Belfanti et al. (2004)
	MpNPR1	*Venturia inaequalis* and *Gymnosporangium juniperi-virginianae*	Increased disease resistance by expression of pathogenesis-related (PR) genes	Malnoy et al. (2007)
	dpo	*Erwinia amylovora*/fire blight	Encoding for an extracellular polysaccharide (EPS)-depolymerase	Flachowsky et al. (2008)
Avocado	*pdf1.2*	–	Encodes an antifungal defensin	Raharjo et al. (2008)
Banana	*rcc2* or *rcg3* class-I rice chitinase gene	*Mycosphaerella fijiensis*/black leaf streak disease	Expression of the chitinase (RCG3) protein	Kovacs et al. (2013)
	ThEn-42 along with *StSy* and *SOD*	*Botrytis cinerea*/Sigatoka disease	Overexpression of fungal endochitinase	Vishnevetsky et al. (2011)
	PhDef1 and *PhDef2* Petunia floral defensins gene	*Fusarium oxysporum*/wilt disease	Encodes antimicrobial peptides 'Defensins'	Ghag et al. (2012)
	ihpRNA-Rep and *ihpRNA-ProRep*	*Banana bunchy top virus* (BBTV)/banana bunchy top disease	Transgenic plants expressing small interfering RNAs targeted against viral replication initiation gene	Shekhawat et al. (2012)

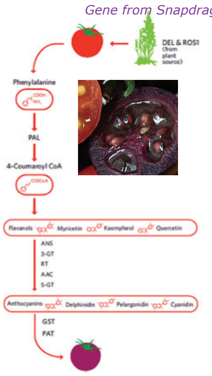
Citrus	*hrpN*	*Xanthomonas axonopodis* pv. *citri*/citrus canker	encodes a harpin protein	Barbosa-Mendes et al. (2009)
	attacin A	*Xanthomonas citri* subsp. *Citri*/Asian citrus canker	Encodes Attacin A, an antimicrobial peptides	Cardoso et al. (2010)
	AtNPR1	*Xanthomonas citri* subsp. *Citri*/citrus canker	Key positive regulator of SAR	Zhang et al. (2010)
	scFv	*Citrus tristeza virus* (CTV)	Overexpression of scFv antibody fragments directed against epitopes of its major coat protein p25	Cervera et al. (2010)
	pthA-nls	*Xanthomonas axonopodis* pv. *citri*/Citrus canker	Encodes three nuclear localizing signals (NLS)	Yang et al. (2011)
	Shiva A and *Cecropin B*	*Xanthomonas axonopodis* pv. *citri*/citrus canker	Encodes a bivalent antibacterial peptide	He et al. (2011)
	Attacin E	*Elsinoe fawcettii*/citrus scab	Encodes Attacin, an antimicrobial peptides acts against fungal pathogen	Mondal et al. (2012)
Grapevine	Stilbene synthase gene (*STS*)	–	Synthesis of the antifungal phytoalexin resveratrol	Fan et al. (2008)
	GFLV movement protein (MPc) gene	Grapevine fanleaf virus (GFLV)	Post-transcriptional gene silencing (PTGS)	Jardak-Jamoussi et al. (2009)
	VpSTS	*Uncinula necator*/powdery mildew	Involvement of stilbene synthase promoter in pathogen- and stress-inducible expression	Xu et al. (2010)
	Chitinase and *b-1,3-glucanase*	*Plasmopara viticola*/downy mildew	Increased activities of chitinase and *b*-1, 3-glucanase in transgenic lines	Nookaraju and Agrawal (2012)
Papaya	PRSV coat protein (*CP*)	*Papaya ringspot virus*	Characterization of Insertion Sites in Rainbow Papaya	Suzuki et al. (2008)
	PRSV and PLDM coat protein (*CP*)	*Papaya ringspot virus* and *Papaya leaf-distortion mosaic virus*	Virus resistance mediated by RNA-mediated posttranscriptional gene silencing (PTGS)	Kung et al. (2009)
	DmAMP1	*Phytophthora palmivora*	Encodes antimicrobial peptides 'Defensins' acts against fungal pathogen	Zhu et al. (2007)
Strawberry	*Chit-42*, or *β-1,3-glucanase*	*Colletotrichum acutatum*/anthracnose crown rot	Transgenic lines showed significantly fewer anthracnose crown rot lesions compared to the controls	Mercado et al. (2007)
	apd	–	Encodes am antimicrobial peptide-D	Qin et al. (2008)

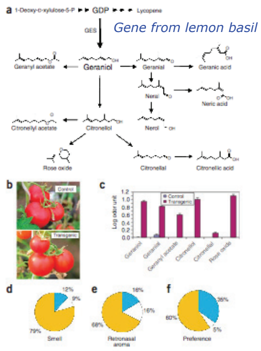
GM tomato with good processing characters



GM tomato with delayed ripening

102





Purple tomato

Flavour enhancing tomato

103

Borer resistant eggplant

PRSV resistant papaya

104

Diagnosis

- Detect the presence of pathogens, contaminants on the plants and on the fruits/vegetable using molecular techniques
- Testing of GM materials
- RT-PCR, real time PCR, hybridisation
- Antisera
- Using minute amount
- Standardisation of the plantation and products

105

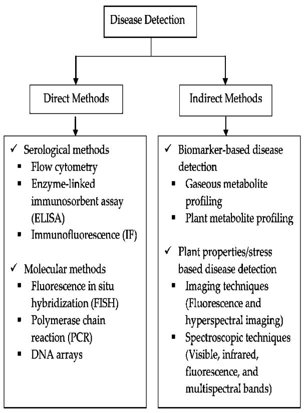


Fig. 1. Methods of plant disease detection.

S. Sankaran et al. / Computers and Electronics in Agriculture 72 (2010) 1–13 3

Table 1
Examples of some studies on plant disease detection using molecular techniques.

Plant/Trees	Pathogen	Type	Molecular method	Reference
Grapevine	*Xylella fastidiosa*	Bacteria	PCR, ELISA	Minsavage et al. (1994)
Citrus	*Candidatus Liberibacter*	Bacteria	PCR	Li et al. (2006)
Citrus	*Xylella fastidiosa, Methylobacterium mesophilicum*	Bacteria	PCR	Lacava et al. (2006)
Citrus	*Citrus tristeza virus*	Virus	PCR, ELISA	Saponari et al. (2008)
Sweet orange	*Candidatus Liberibacter asiaticus*	Bacteria	Isothermal, chimeric primer-initiated amplification of nucleic acids + cycling probe technology	Urasaki et al. (2008)
Rice	*Burkholderia glumae*	Bacteria	Fluorescence PCR	Fang et al. (2009)
Potato	*Candidatus Liberibacter solanacearum*	Bacteria	PCR	Li et al. (2009a)
Citrus	*Citrus leaf blotch virus*	Virus	PCR	Ruiz-Ruiz et al. (2009)
Tomato	*Pepino mosaic virus*	Virus	PCR, ELISA	Gutiérrez-Aguirre et al. (2009)
Almond	*Candidatus Phytoplasma prunorum*	Bacteria	PCR	Yvon et al. (2009)

F. Martinelli et al.

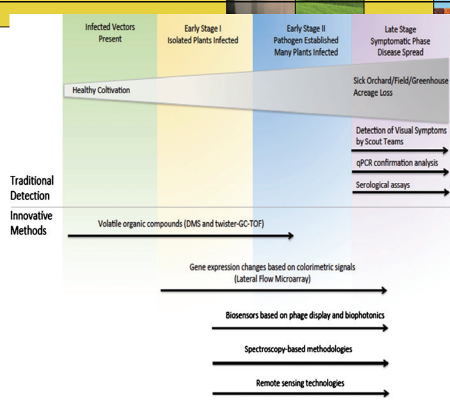
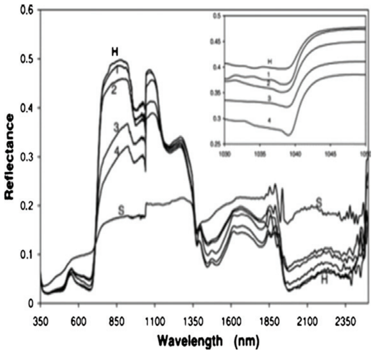


Fig. 3 Traditional and innovative methods. Their timing of use during plant disease progression was indicated. Four disease stages were considered

Agron. Sustain. Dev.
DOI 10.1007/s13593-014-0246-1

F. Martinelli et al.

Fig. 4 Field reflectance spectra for healthy tomatoes plants (H) and plants infected with late blight disease increasing severity (from 1 to 4). S is the average spectrum for soil. The insert is an enlarged view of the abrupt changes at approximately 1,040 nm (from Zhang et al. 2003) (courtesy of the International Journal of Applied Earth Observation and Geoinformation, edited by Elsevier)



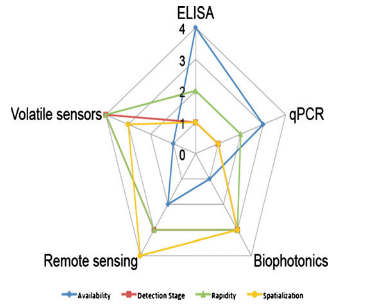


Fig. 7 Comparison of methods for plant disease detection (PDD). The qualitative scales indicate: 1 poor, 2 fair, 3 good, and 4 very good. The categories evaluate individual techniques with respect to: (i) Availability—ease of use, availability of equipment, and cost; (ii) detection stage—when infections can be detected (4 infected vectors present, 3 isolated infected plants, 2 many infected plants, and 1 symptomatic stage disease has spread over the cultivated area); (iii) speed—total time required between collection of field data and the delivery of results (thus includes sample collection, preparation, and testing); (iv) spazialization—the potential to spatialize results (4 input data already carried out in a spatialized dimension, 3 data easily spatializable, 2 data difficult to spatialize, and 1 data not subject to spatialization); and (v) reliability—effective accuracy of results

Adulteration detection

- Trade food and agricultural commodities
- Black pepper, chilli, turmeric
- Destroy reputation and trade
- DNA based techniques

111

TABLE 1: Types of Adulterants and their examples

TYPE	FEW EXAMPLES OF SUBSTANCES ADDED
Intentional Adulterants	
• Physical adulterant	Sand, marble chips, stones, mud, other filth, talc, chalk powder, water, mineral oil
• Biological adulterant	Papaya seeds in black pepper, Argemone seeds in mustard seed etc.
Incidental Adulterants	
• Natural adulteration	Toxic varieties of pulses, mushrooms, green and other vegetables, fish and sea foods
• Non natural adulteration	Pesticide residues, tin from can, droppings of rodents, larvae in foods
Metallic Contaminants	Arsenic from pesticides, lead from water, mercury from effluent, from chemical industries, tins from cans

Microbial contaminant	
▪ Bacterial	*Bacillus cereus, Clostridium botulinum toxins, Clostridium perfringens (welchii), Salmonella, Shigella sonnei, Staphylococcus aureus, Streptococcus pyogenes*
▪ Fungal	*Aspergillus flavus* (aflatoxin), *Claviceps purpurea* (Ergot), *Fusarium sporotrichiodies, Penicillium islandicum*
▪ Parasiticus	*Trichinella spiralis, Ascaris lumbricoides, Entamoeba histolytica, Ancylostoma duodenale* (hookworm)

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Table 2. Common adulterants in some of the major traded spices

Commodity	Adulterants	
	Chemical / earthy material	Biological
Black pepper berries (*Piper nigrum*)	mineral oil	Dried papaya seed (*Carica papaya*); wild *Piper* Spp. (*P. attenuatum* and *P. galeatum*); fruits of *Lantana camara* and *Embelia ribes*; seeds of *Mirabilis jalapa*; berries of *Schinus molle*; exhausted black pepper; light berries, stems and chaff of black pepper.
Black pepper powder	Dye	Powdered papaya seed; wild *Piper* berries; *Lantana camara*; *Embelia ribes*; *Mirabilis jalapa seeds*; *Schinus molle* berries; exhausted black pepper and light berries; starch from cheaper source
Chilli fruits (*Capsicum annuum*)	Dyes, mineral oil	-
Chilli powder	Dye- coal tar red, sudan red, para red; vanilyl-n-nonamide; Mineral oil; talc powder; brick powder; salt powder.	Powdered fruits of 'Choti ber' (*Ziziphus nummularia*); red beet pulp; almond shell dust; extra amounts of bleached pericarp, seeds, calyx, and peduncle of chilli; starch of cheap origin; tomato wastes.
(*Turmeric power. Curcuma longa*)	Dye- Metanil Yellow, Orange II lead chromate; chalk powder; yellow soap stone powder.	Wild *Curcuma* spp- *C. zedoaria* Rosc or 'yellow shotti' syn. *C. xanthorrhiza* Roxb. ('Manjakua') or *C. malabarica*; starch from cheaper source; saw dust.

Dhanya and Sasikumar, 2010

115

Table. 1. Adulterant/contaminant detection and authenticity assessment of plant derived food and agricultural commodities using DNA based techniques.

Application	Technique	Target gene	Reference
Detection of cashew husk (*Anacardium occidentale L.*) adulteration in tea [*Camellia sinensis* (L.) samples	Species-specific PCR	ITS of 5S rRNA	(92)
Differentiation of 'Arabica' and 'Robusta' coffee beans	PCR-RFLP	chloroplastic genome	(93)
Detection of rhubarb yogurt in raspberry yogurt	PCR, sequencing	chloroplast *rbcL*	(51)
Detection of mei (*Prunus mume*) and plum (*Prunus salicina*) adulteration in preserved fruit products	Specific PCR	Ribosomal ITS1	(95)
Authenticity testing of raw rice materials in rice-based food product	SSR	Microsattelite DNA	(57)

Article J. Agric. Food Chem., Vol. 59, No. 2, 2011 515

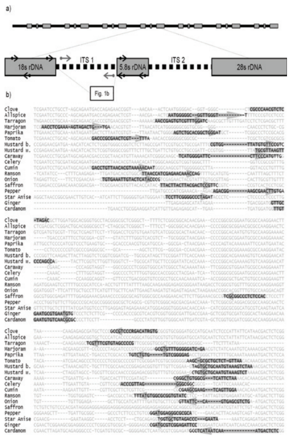


Figure 1. rDNA sequences and primer binding sites. (**a**) Gray boxes: rDNA genes (18s, 5.8s, and 28s), dotted lines ITS 1 and 2; arrows: primer binding sites (gray, specific; black, universal). (**b**) Sequence alignment of ITS1 created with ClustalX v. 2.0.9 (20) with primer binding sites. Gray arrows with black font: primer binding sites. Reverse primer binding sites for allspice and celery are located in ITS2; they are not shown in **b**.

Table 3. Specific Primer Sets

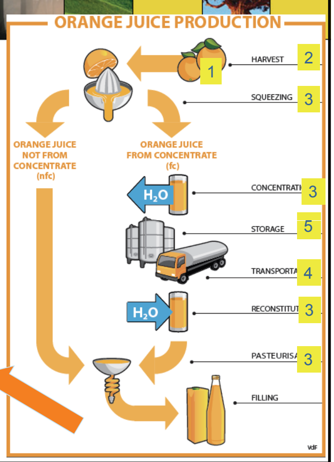
spice	forward primer 5′→3′ reverse primer 5′→3′	length (bp)	accession[a]	abbreviation
allspice[b] (*P. dioica*)	AATGGGGGCGGTTGGGTT CCCTGGCCGTGGCTTC[c]	333	AM234081	All
black mustard[b] (*Brassica nigra*)	CGTGGTTATGTGTTCCGTC TTAGACTTTACATTGCAGCACTA	184	DQ340645	MuB
caraway[b] (*C. carvi*)	GGGATTCCTTCCCATGTTG TTAGAATGACGCCACAGCC	151	AF077878	Car
cardamom (*E. cardamomum*)	TTGTGAATGTGTCAACGCGC GAGAGTCATTTGATTATGAGGC	163	GQ166167	Card
celery[b] (*Apium graveolens*)	ACCCGTTAGGGGCGGC CTCCTTAGATGACACAATTACG[c]	~370[c]	U30552.1 U30553.1[c]	Cel
clove (*S. aromaticum*)	CGCCCAACGTCTCTAGAC CACCATGTCTGGGACGGC	142	EF026622	Clo
cumin[b] (*Cuminum cyminum*)	GACCTGTTAACACGTAAAAACAAT TCCAACTGACTTCGCTTCG	190	CCU78362	Cum
ginger[b] (*Zingiber officinale*)	GTTGCGAATGCGTGAATGTG GGAATCTCCGACGCATCG	157	DQ064590	Gin
marjoram (*Origanum majorana*)	AACCTCGAAAAGTAGACTGTGA TCGATCCCCCAAACACGC	207	GQ166166	Maj
onion[b] (*Allium cepa*)	TGTGAAATTGTACTCATACCCG CAGACGCTCACTGGAATAAC	215	AJ411944	Oni
paprika (*Capsicum annuum*)	AGTCTGCACGGCTGGGAT CTCCCCGACACACAGACA	169	GQ166165	Pap
pepper[b] (*Piper nigrum*)	AGACGGAAGCGAACTTGTGA TGCGCGCCCTCCATCC	164	EF060077	Pep
ramson[b] (*A. ursinum*)	TTAACCATCGAGAACAAACCAG GATACACCGCGCCACATAAA	184	AJ412744	Ram
saffron[b] (*Crocus sativus*)	TTACTTACTTACGACTCCGTTC GTGGAGAGGGCCGCGA	128	DQ094185	Saf
star anise[b] (*I. verum*)	TCCTTCGGGGCCCTAGAT TATTCGGGTCTACAGCACCA	182	AF163724	StA
tarragon[b] (*A. dracunculus*)	AACCGAGTGTCGTTTGGATC CGGGGCTACACGAAACGA	173	AF045401	Tarr
tomato[b] (*Lycopersicon esculentum*)	GACCCGCGAACTCGTTTTA TTAACAGAGCAGCGCGCTT	196	AF244747	Tom
white mustard[b] (*Sinapis alba*)	TGCGTTAAGTTCCCAGCCA AGACTTTACATTGCAGCACAG	169	AY722486	MuW

[a] Accession number of sequence used for primer design. [b] Primer sets used to optimize PCR conditions. [c] Reverse primer hybridizes in ITS2.

Discussion and conclusion

① Fruit and vegetable products
② Harvest
③ Processing
④ Package and transport
⑤ Storage
⑥ Consumption

Post-harvest



Biotechnology

- Plant tissue culture
- Molecular marker
- Genetic engineering
- Disease monitoring
- Adulteration detection

(Plant tissue culture, Molecular marker, Genetic engineering: Premium line)
(Genetic engineering, Disease monitoring, Adulteration detection: Customer satisfaction)

Bottlenecks

- Not every techniques are available (at all, in your countries, legal issues etc)
- Some techniques require trained scientists, well equipped laboratories, financial support

Opportunities

- **Each country has varieties of fruits and vegetables that can export AND increase the value after harvest --- but--- Due to the limit resources identification of champion fruits/vegetables might be important**
- **Some thought is needed from plantation, harvest, processing, packaging and manufacture whereby biotechnology can play important roles**

123

- **Other technology including material science, computer science, engineering and food science are also needed to improve/increase the value of our fruits and vegetables**
- **Co-operation (institutes/regions/global)**

Post-test (please write your name and country)

- Descript one technique of biotechnology that could be useful to you and why