Biotechnology and its applications for fruit and vegetable products

PARICHART BURNS

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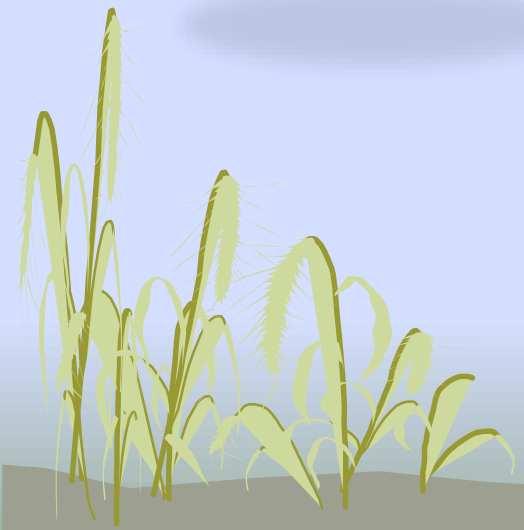
Outline

1 INTRODUCTION

2 BIOTECHNOLOGY

3 CONCLUSION &DISCUSSION



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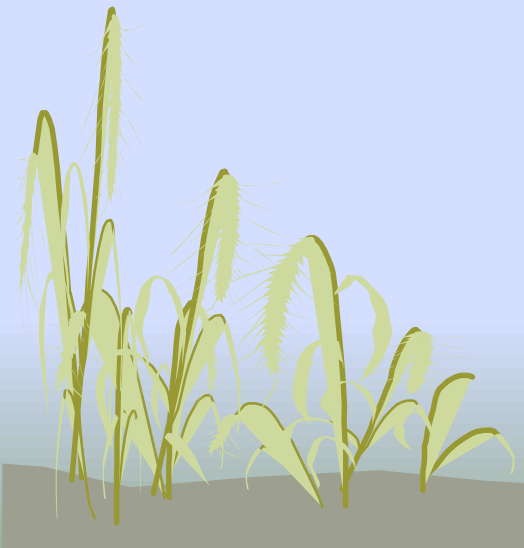
INTRODUCTION

HISTORY OF AGRICULTURE AND BIOTECHNOLOGY



Agriculture proposed origin

15,000 years ago | 10,000 | 5,000 | 0 Present Day

Domesticated Crops

Cultivation

Possible Cultivation

ComPAg

http://www.ucl.ac.uk/archaeology/research/directory/compag-fuller

pottery

West Africa (Pearl millet)

livestock

cultivation

sedentism

agricultural landscape

Levant Near East (Wheat & Barley)

sedentism

cultivation

livestock

agricultural landscape

pottery

India Savanna (Millets)

pottery

cultivation

livestock

sedentism

agricultural landscape

India Ganges (Rice)

pottery

cultivation

sedentism

livestock

agricultural landscape

North China (Millets)

pottery

cultivation

sedentism

livestock

agricultural landscape

South China Yangtze (Rice)

pottery

cultivation

sedentism

livestock

agricultural landscape

- 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

Early biotechnology

- Based on natural microorganisms (such as yeast) or biomolecules
- Require fermentation/incubation period
- Basic equipment/tool
- Local consumption and export

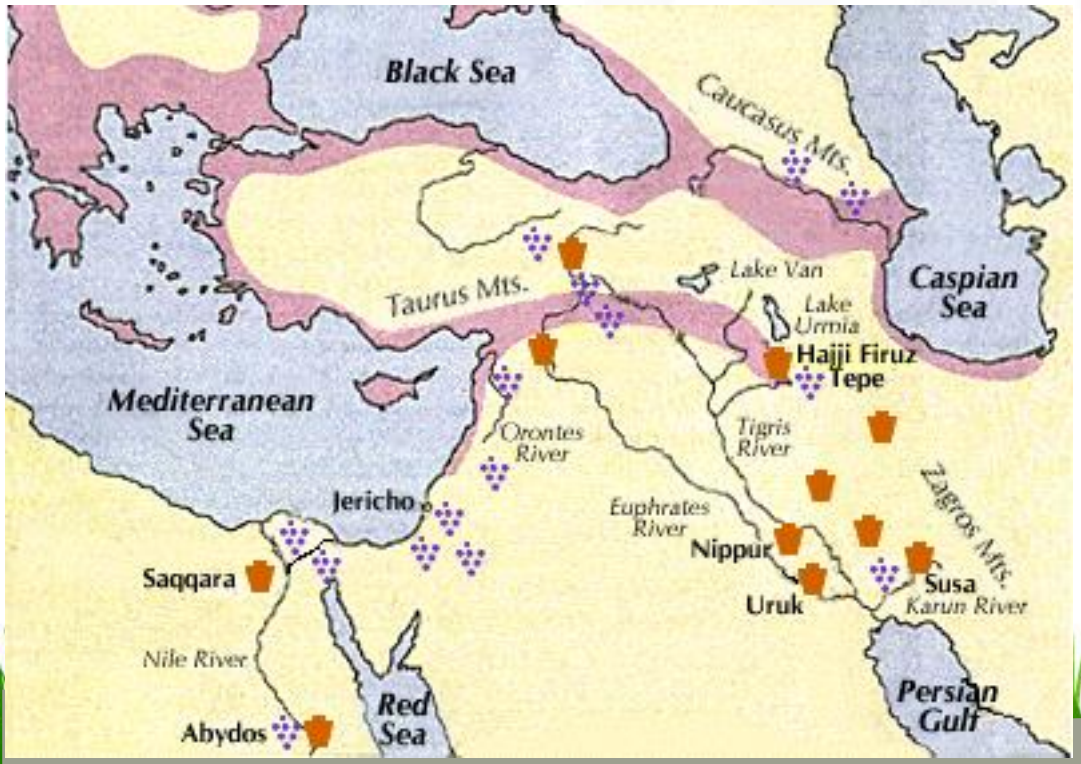
BREAD

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 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

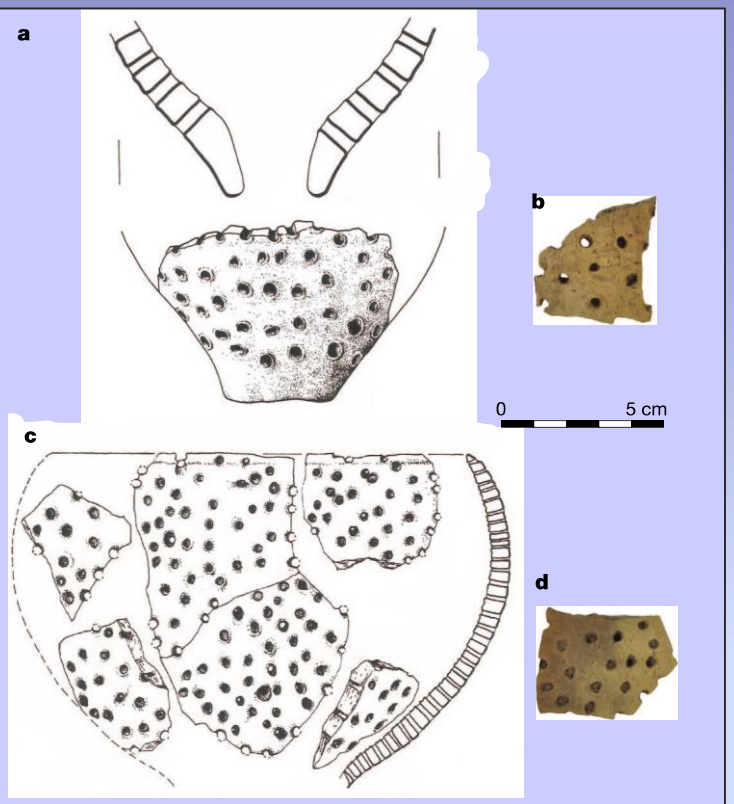


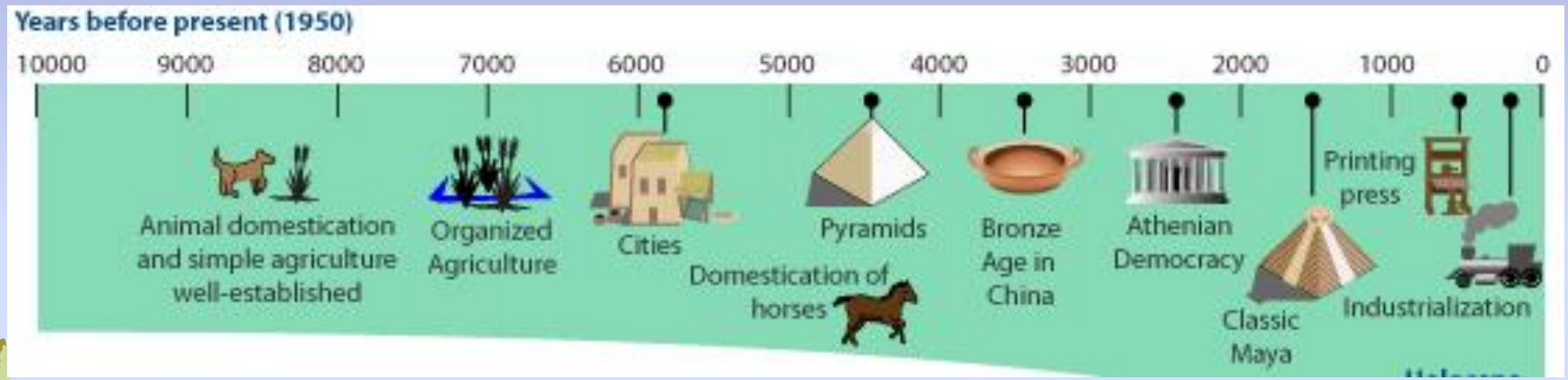
Figure 1 | Drawings of representative reconstructed sieve vessels and photographs of specific sieve fragments from the region of Kuyavia submitted to lipid residue analyses. a, b, KUY0750, from Brześć Kujawski site 3. **c**, **d**, KUY0757 from Smólsk site 4. The typology of the sieve vessels is comparable to those used by modern-day cheese producers (Supplementary Fig. 1). Drawings used with permission from ref. 20.

2013

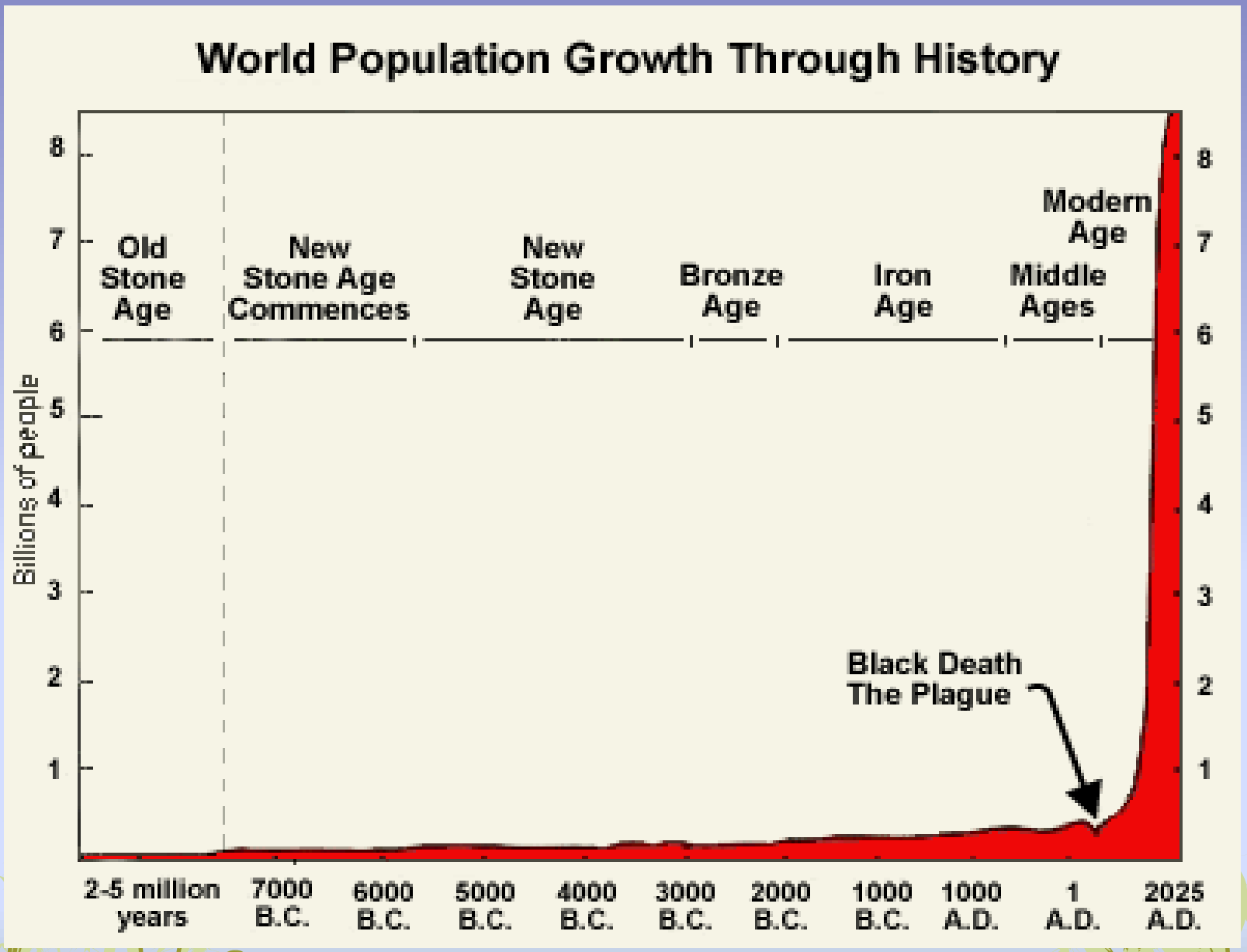
- >6,000 year old
- Found in Europe, Africa, Asia

Agriculture

Industry revolution



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.

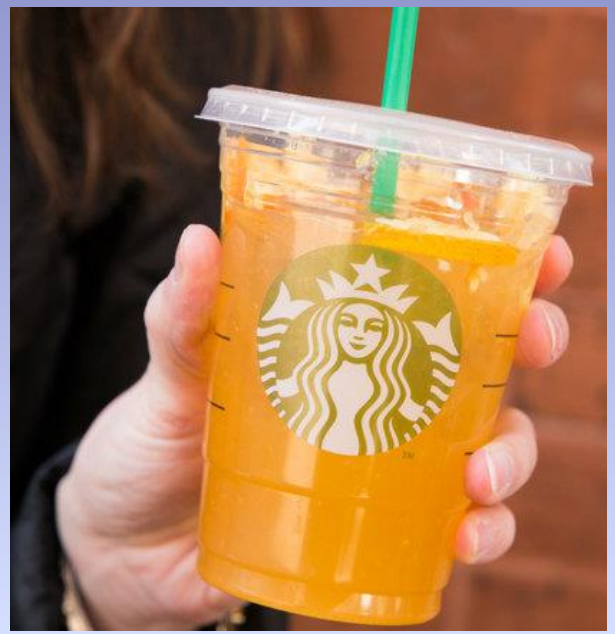


http://phys.org/news/2010-06-humans-extinct-years-eminent-scientist.html

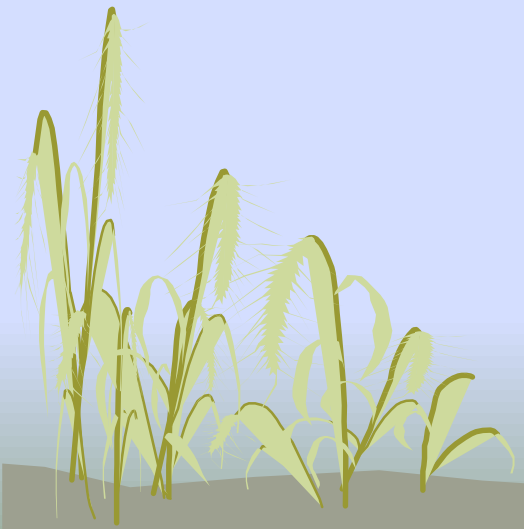
Modern ages

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


Green Giant
Sweet Peas
KEEP FROZEN
Sweet Peas



STEPS IN FRUIT AND VEGETABLE HANDLING



① Fruit and vegetable products

② Harvest

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③ Processing

④ Package and transport

⑤ Storage

⑥ Consumption

Post-harvest

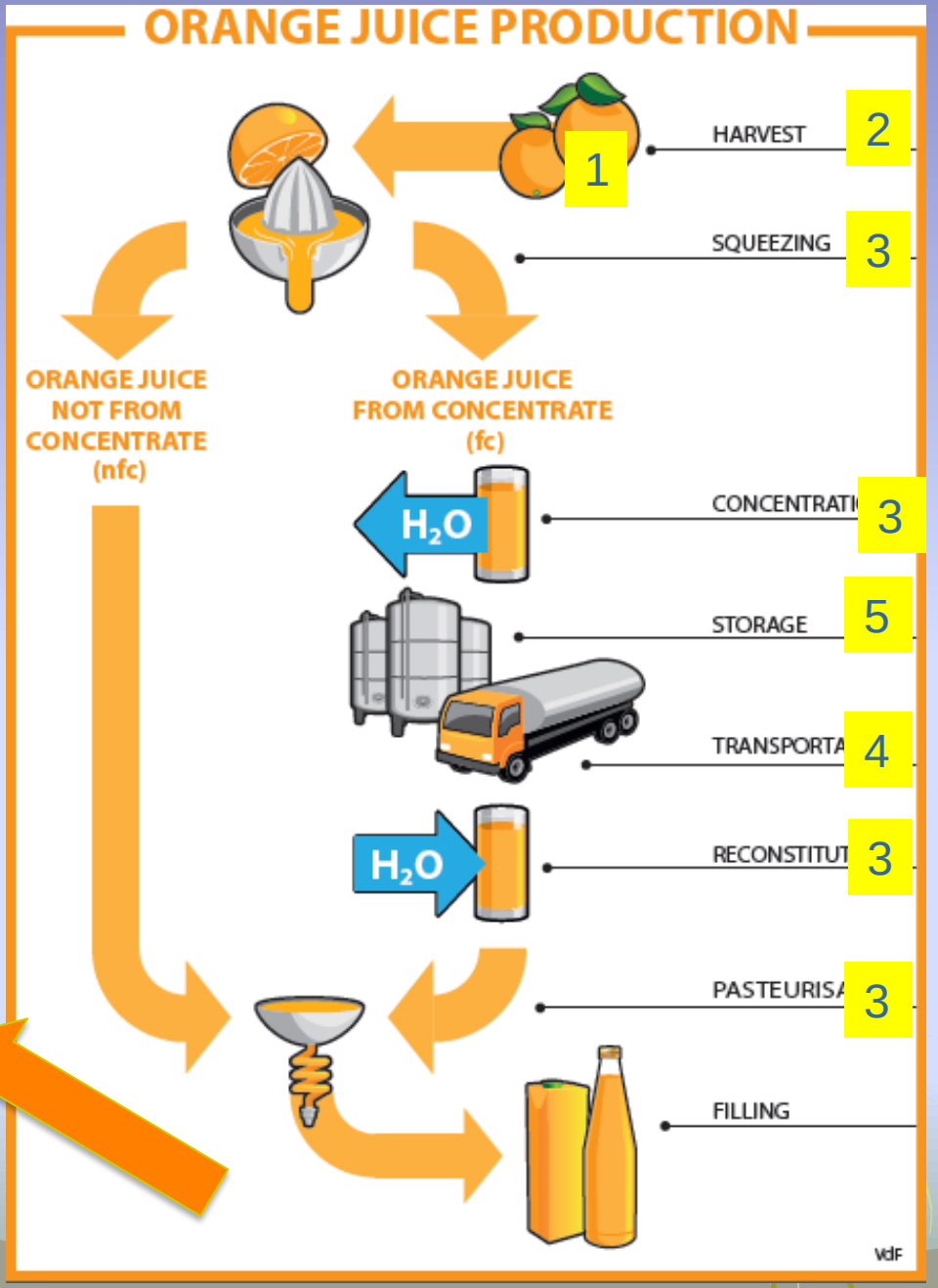


Table 1. Comparison between properties of cereals and roots and tubers regarding their storage capacity
(Source: FAO, 1984, quoted by Knoth, J., 1993)

Non-perishable food crops	Perishable food crops
Harvest manly seasonal, need for storage of long duration	Possibility of permanent or semi-permanent production, needs for short-term storage
Preliminary treatment (except threshing) of the crop before storage exceptional	Processing in dried products as an alternative of the shortage of fresh products
Products with low level of moisture content (10-15 percent or even less)	Products with high level of moisture in general between 50-80 percent
Small "fruits" of less than 1 g	Voluminous and heavy fruits from 5 g to 5 kg or even more
Respiratory activity very low of the stored product, heat limited	High or even very high respiratory activity of stored products inducing a heat emission in particular in tropical climates
Hard tissues, good protection against injuries	Soft tissues, highly vulnerable
Good natural disposition for storage even for several years	Products easily perishable, natural disposition for storage between some weeks up to several months (strong influence of the varieties)
Losses during storage mainly due to exogenous factors (moisture, insects or rodents)	Losses due partly to endogenous factors (respiration, transpiration, germination) and partly to exogenous factors (rot, insects)

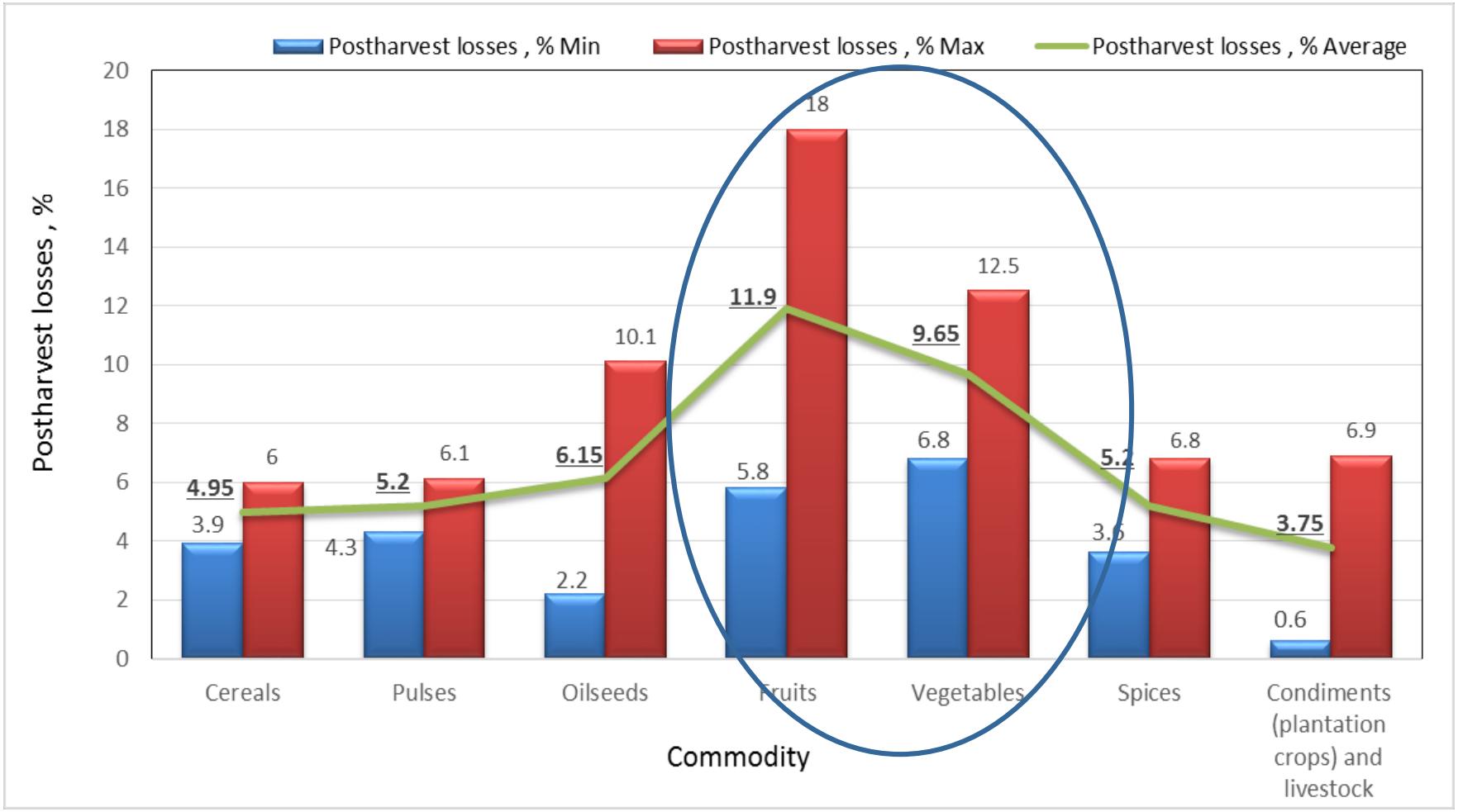


Fig. 1 Harvest and post-harvest losses in different commodities from nation scale quantitative assessment in India 2011.

https://s3-eu-west-1.amazonaws.com/...u.../**PostharvestS**ituationand**Losses**inIndia.pdf

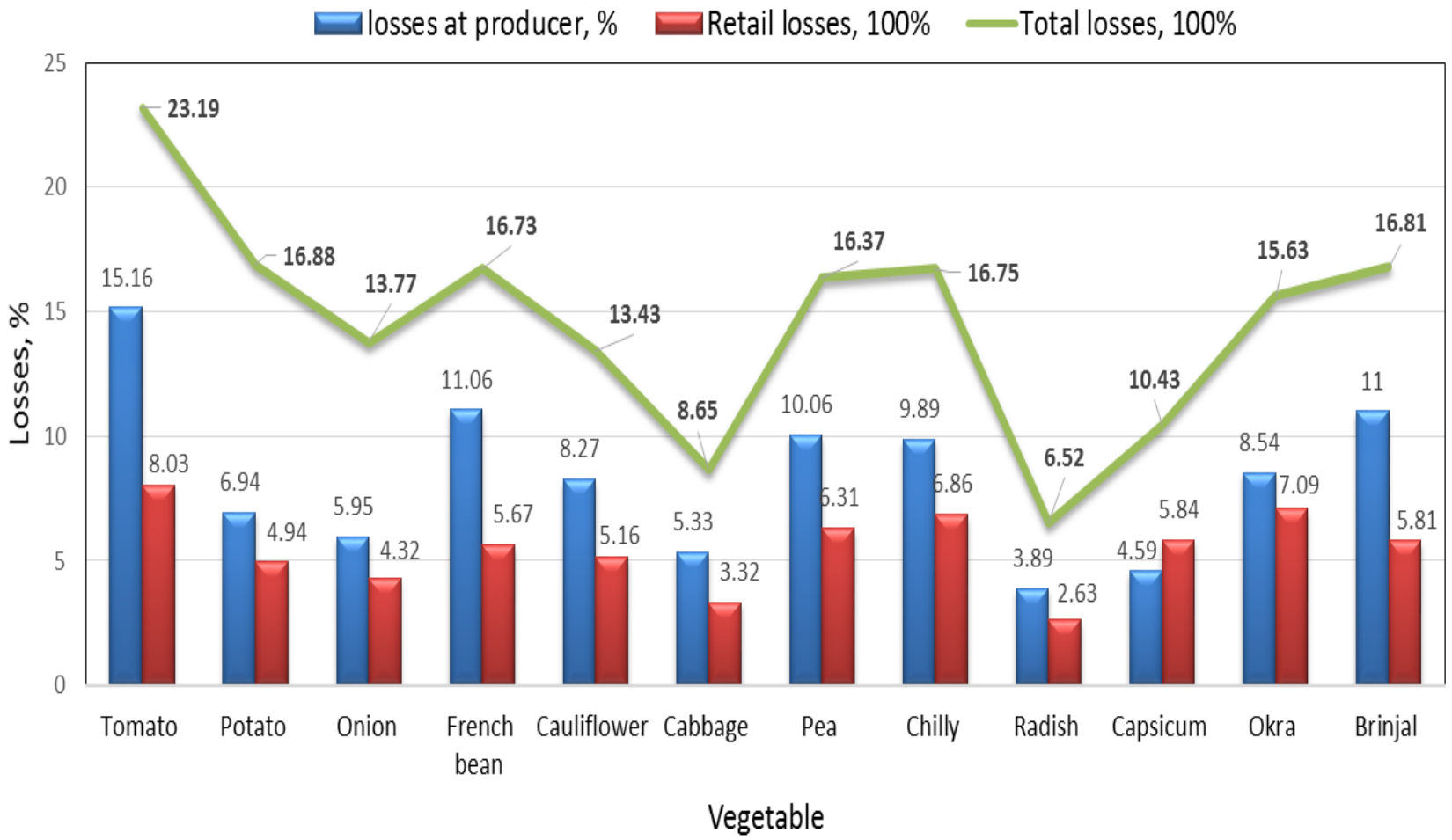


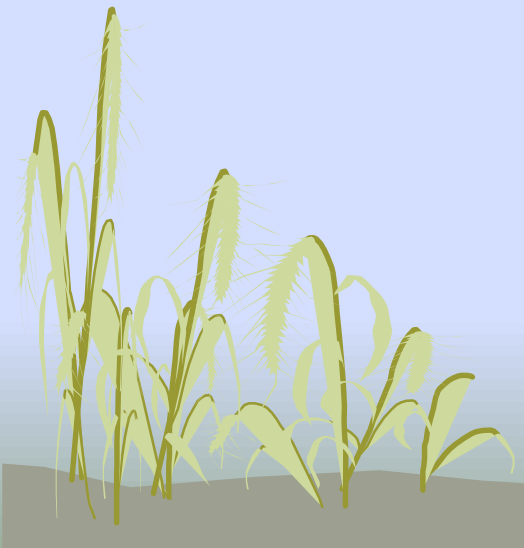
Fig 2 Post-harvest losses of different 12 kind of vegetable at producer and retail levels for 12 major vegetables in Uttarakhand

https://s3-eu-west-1.amazonaws.com/...u.../**PostharvestS**ituationand**Losses**inIndia.pdf

Types of postharvest losses

- Biophysical loss
  - Spoilage
  - Breakage
- Nutrient loss
- Economic loss
  - Financial loss
  - Market force loss

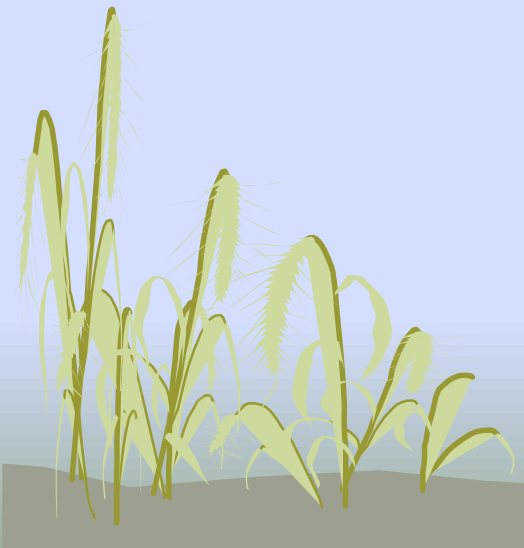
WHAT DO WE WANT FROM BIOTECHNOLOGY ?



- **Premium line**
  - Use less water, fertilizer = starting cost reduction
  - Reliable production (timing, quantity and quality) = more profit
  - High nutritional value = increase value
  - Slow senescence = waste reduction, more profit
  - Products which is robust and have flexibility to manufactured into high value products

- **Maintenance of premium lines**
  - Good seeds
  - Reliable cutting/stocks
- **Detection of impurity**
  - Chemical contamination
  - Microbial contamination
  - Other contamination such as soil
  - Adulteration

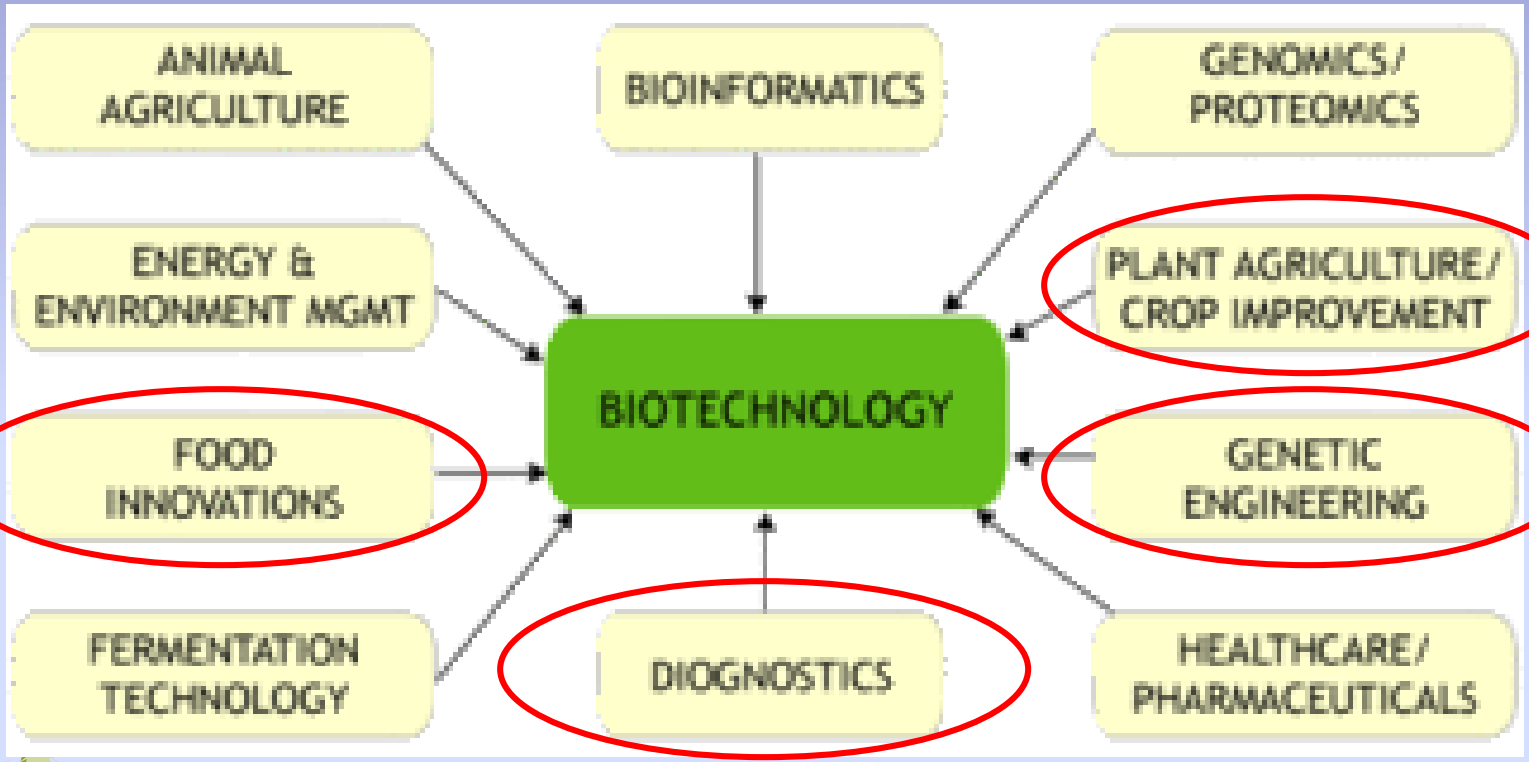
HOW DOES BIOTECHNOLOGY HELP REDUCING POSTHARVEST LOSSES ?



(Modern) Biotechnology

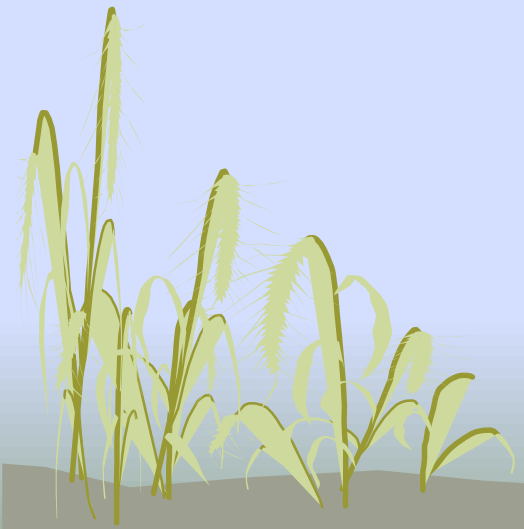
(MODERN) BIOTECHNOLOGY

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.

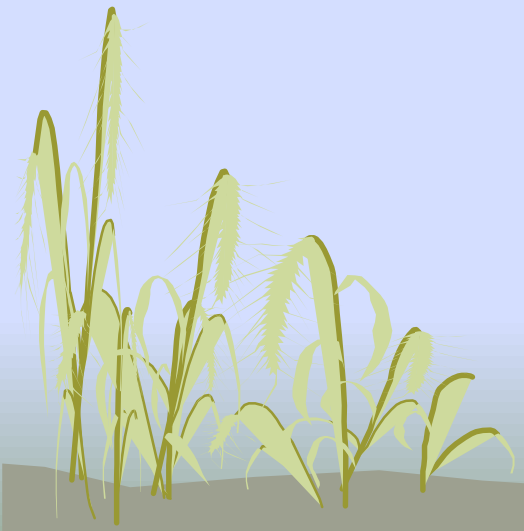

ANIMAL AGRICULTURE
BIOINFORMATICS
GENOMICS/ PROTEOMICS
ENERGY & ENVIRONMENT MGMT
PLANT AGRICULTURE/ CROP IMPROVEMENT
BIOTECHNOLOGY
FOOD INNOVATIONS
GENETIC ENGINEERING
FERMENTATION TECHNOLOGY
DIOGNOSTICS
HEALTHCARE/ PHARMACEUTICALS

Plant biotechnology

- Plant tissue culture
- Molecular marker



PLANT TISSUE CULTURE

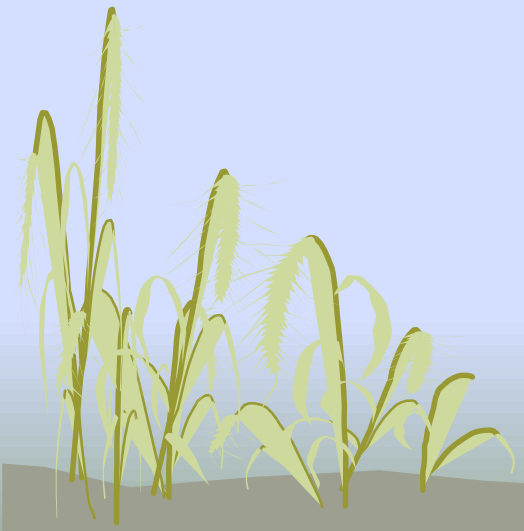


Plant tissue culture

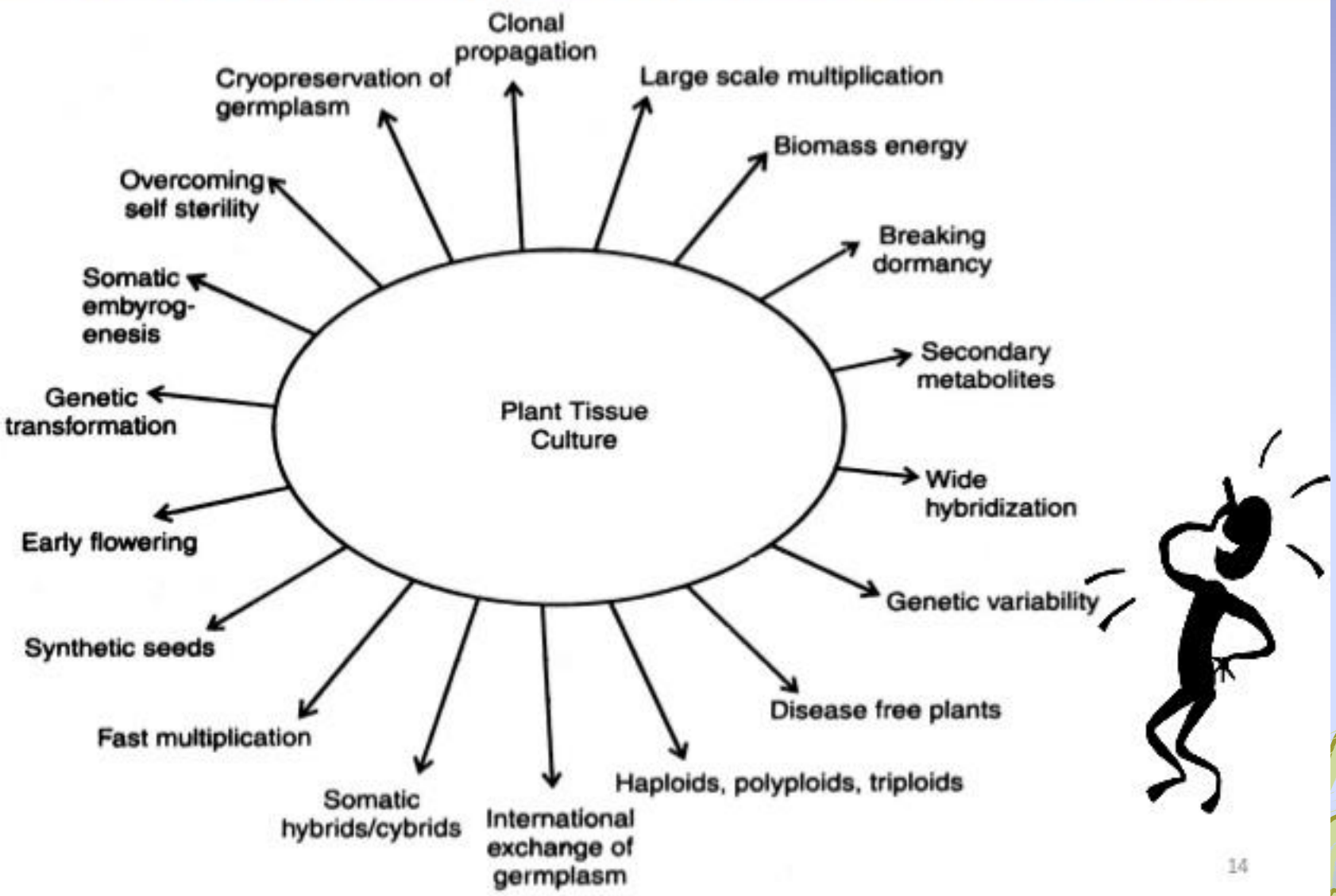
- Generate plantlets/organs/un-differentiate tissues in vitro
- Homogenise in plant developmental stages and strength (synchronize production)
- With/without seeds

Plant tissue culture

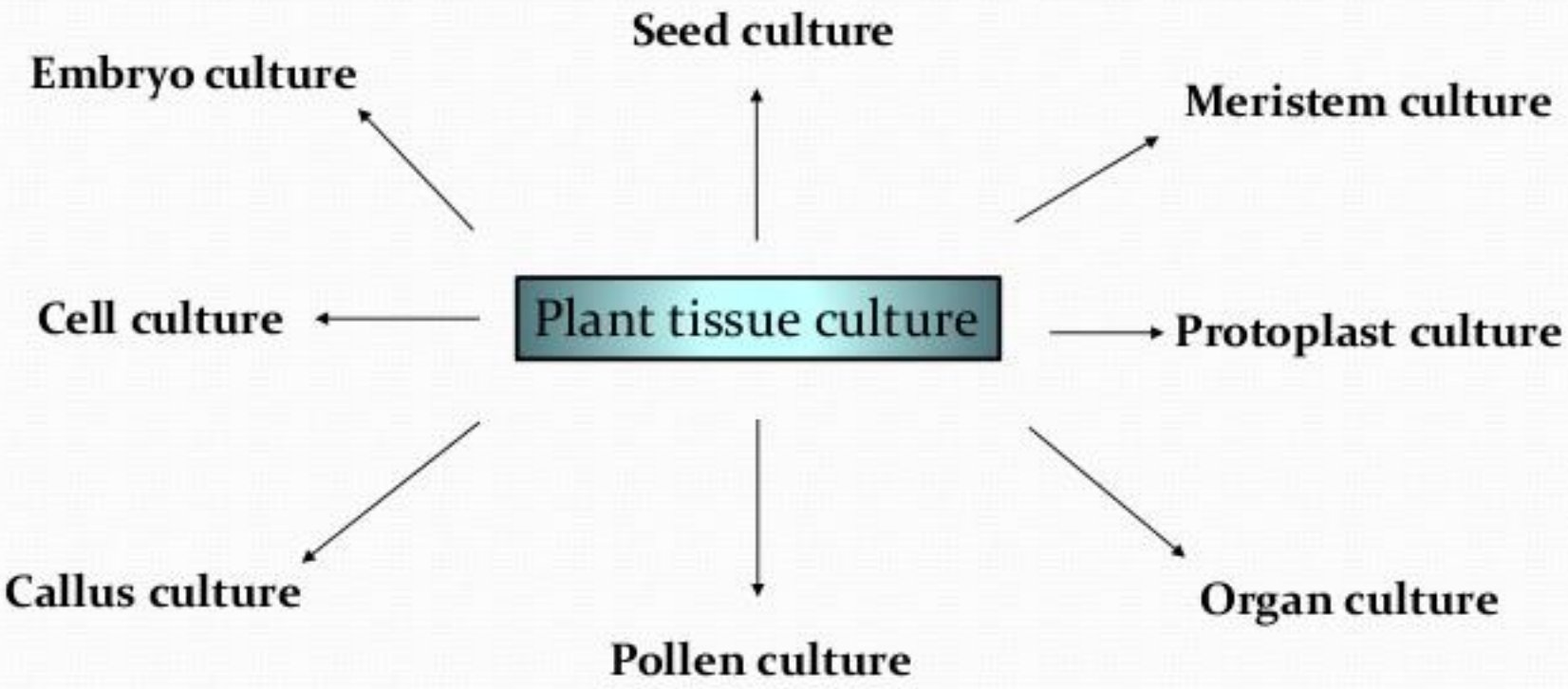
- Using synthetic and complex media
- Sterilize condition
- Disease free

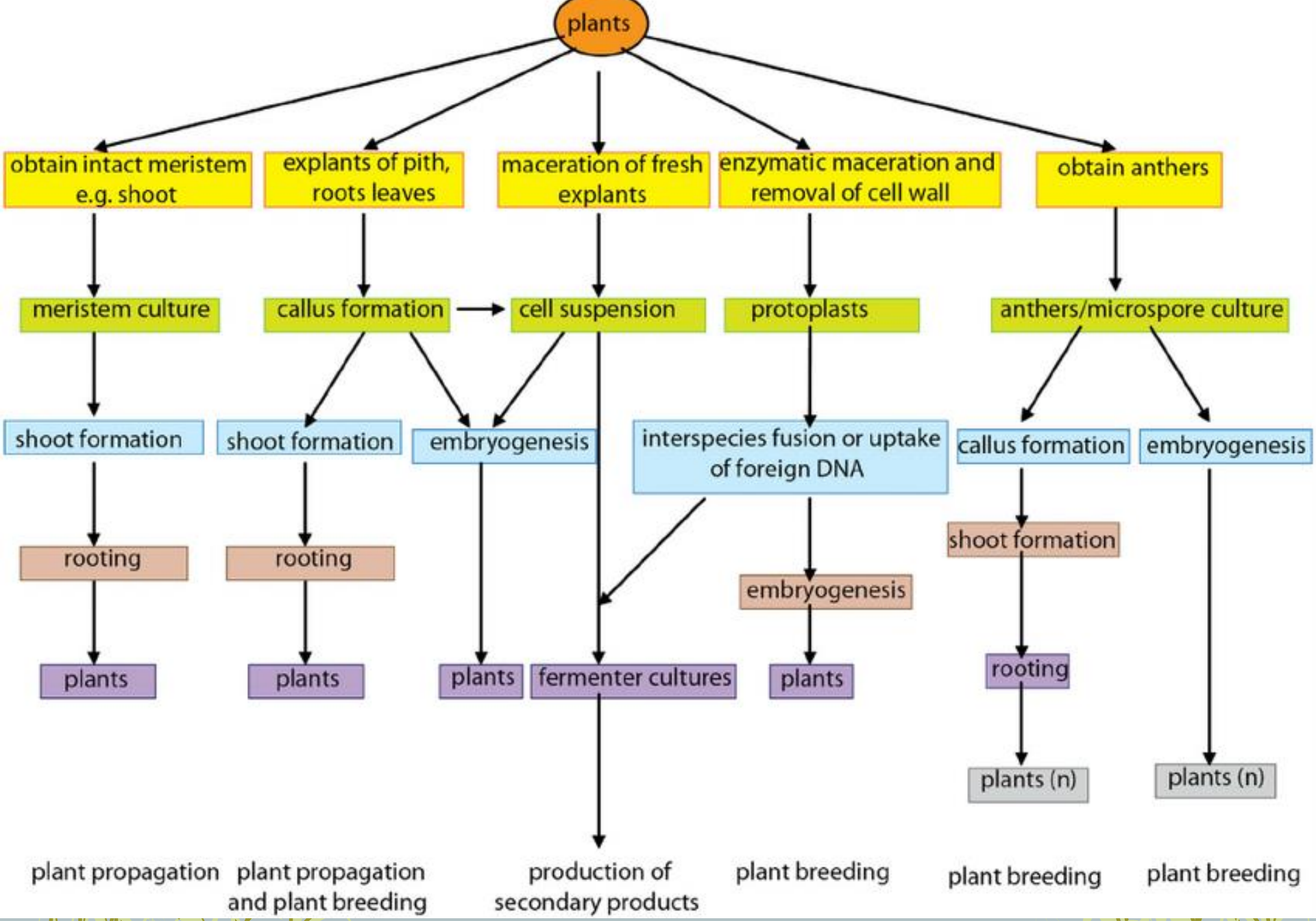


Applications of tissue culture to plant breeding

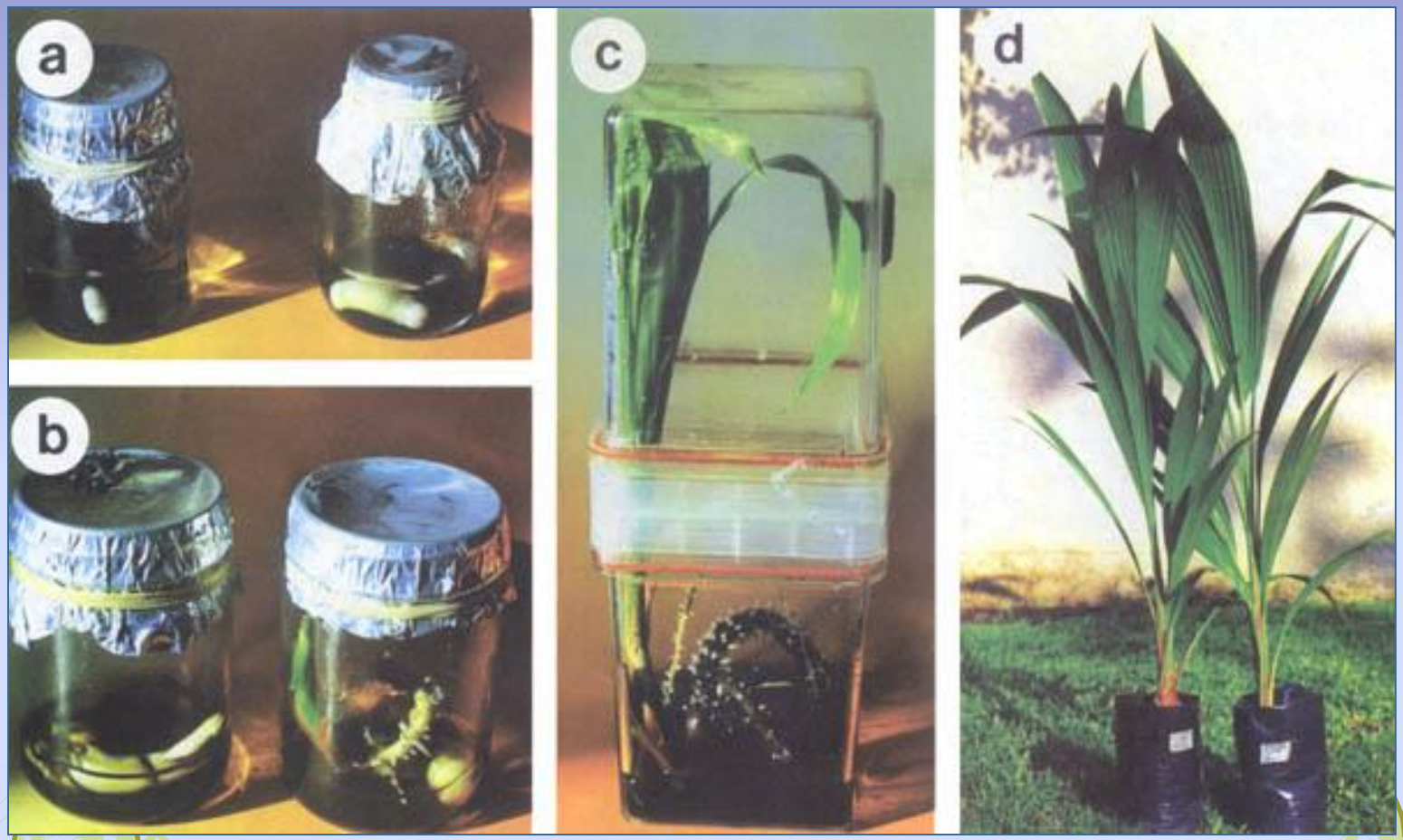


TYPES OF PTC





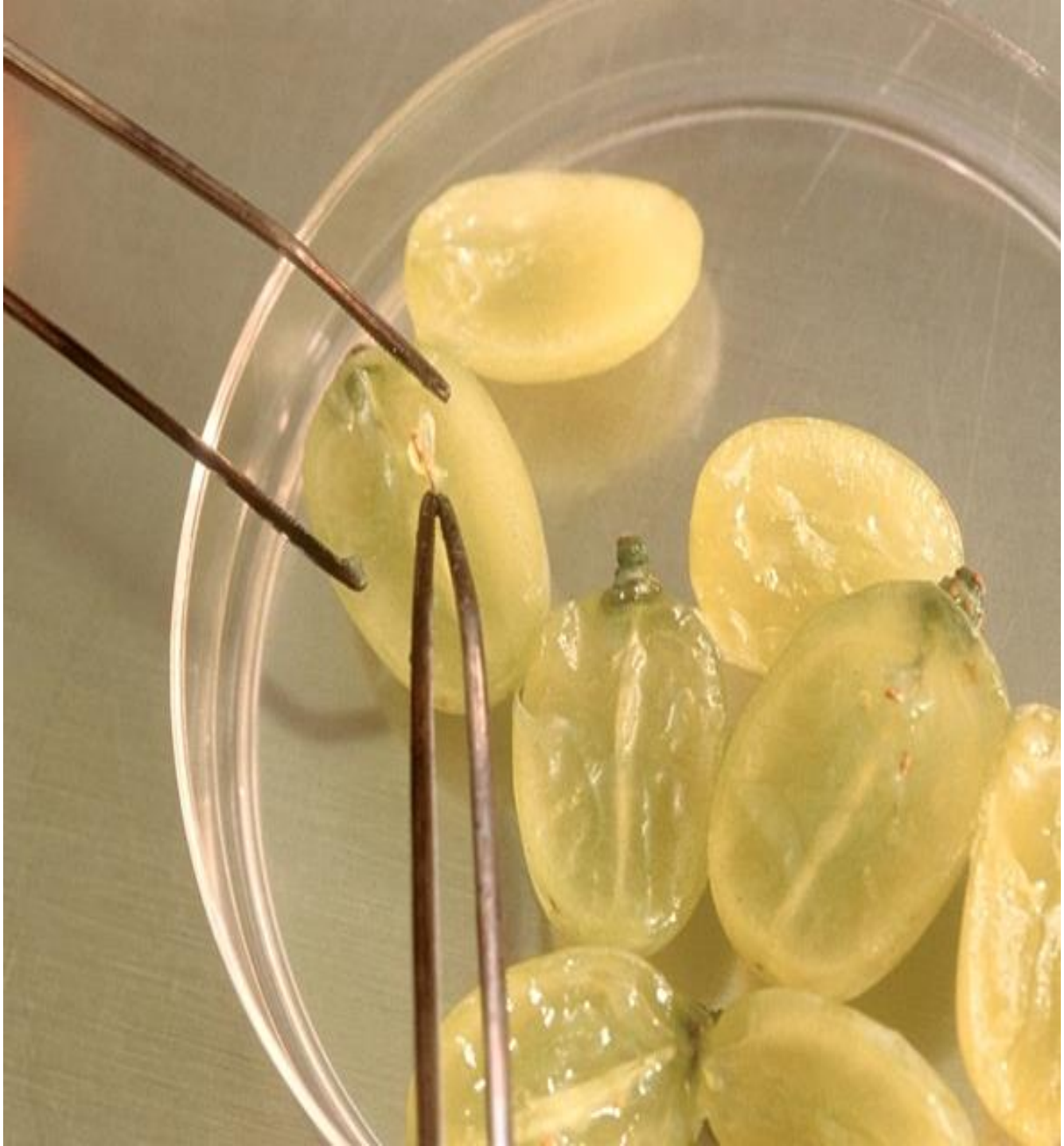
https://www.researchgate.net/profile/Moniruzzaman_Mr/publication/284131355/figure/fig4/AS:309101377671170@1450706919402/Fig-4-The-major-area-of-plant-cell-and-tissue-culture-and-its-application.png



Coconut tissue culture

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

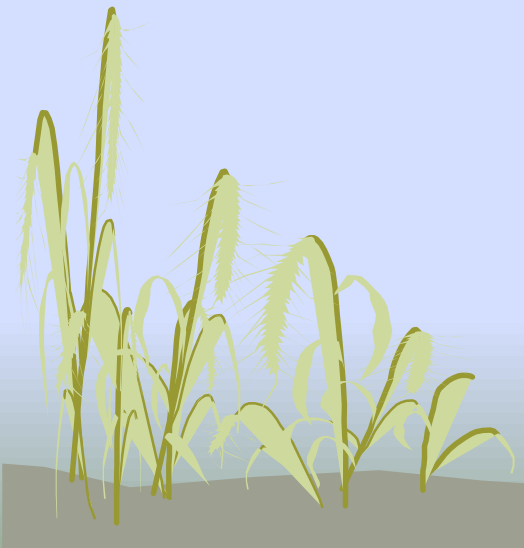



Capsicum frutescens
COO-904


C. frutescens x C. annuum (KA)
F1 Obtained
Through
Embryo-Rescue


Capsicum annuum
Var:-kashi Anmol

MARKER ASSISTED SELECTION (MAS)

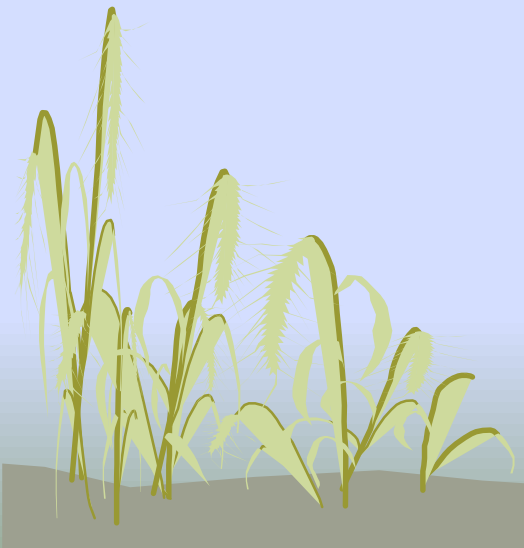


- **A process whereby a marker**

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)

MAS

- Morphological marker
- Biochemical marker
- Cytological marker
- Molecular marker



Morphological markers

- the presence or absence of awn, leaf sheath coloration, height, grain color, aroma of rice

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

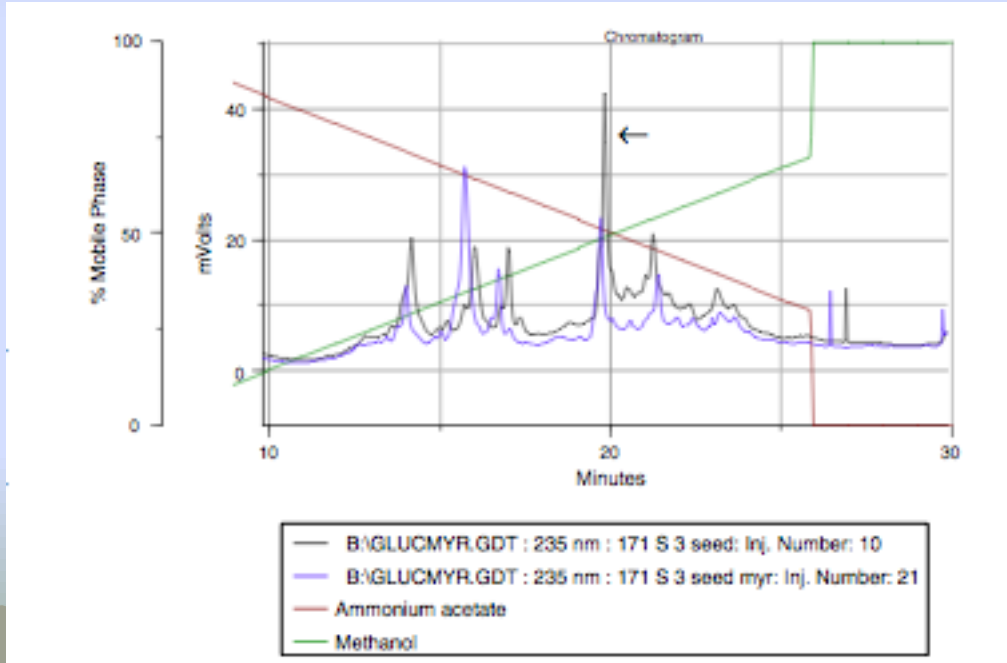
Taste

Flesh aroma

Biochemical markers

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

nicotianamine synthase



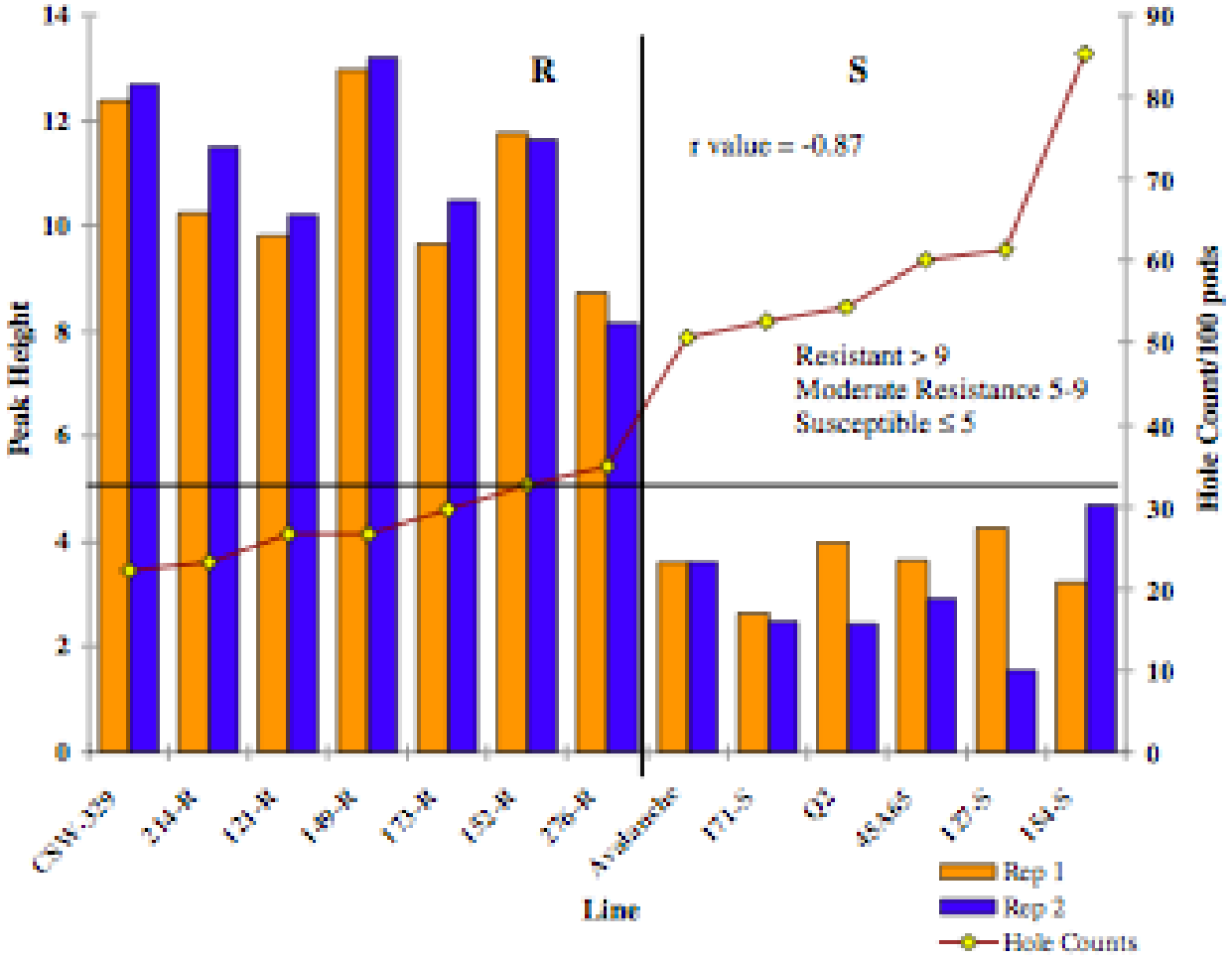


Fig. 3 Comparison between replicated standardized peak height concentrations in upper cauline leaf extracts and larval exit hole counts from the field trial in 2007. Data are organized by ascending larval exit hole counts. The *r* value at −0.87 shows a strong significance difference between R and S

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

Euphytica (2009) 170:297–308

S

R

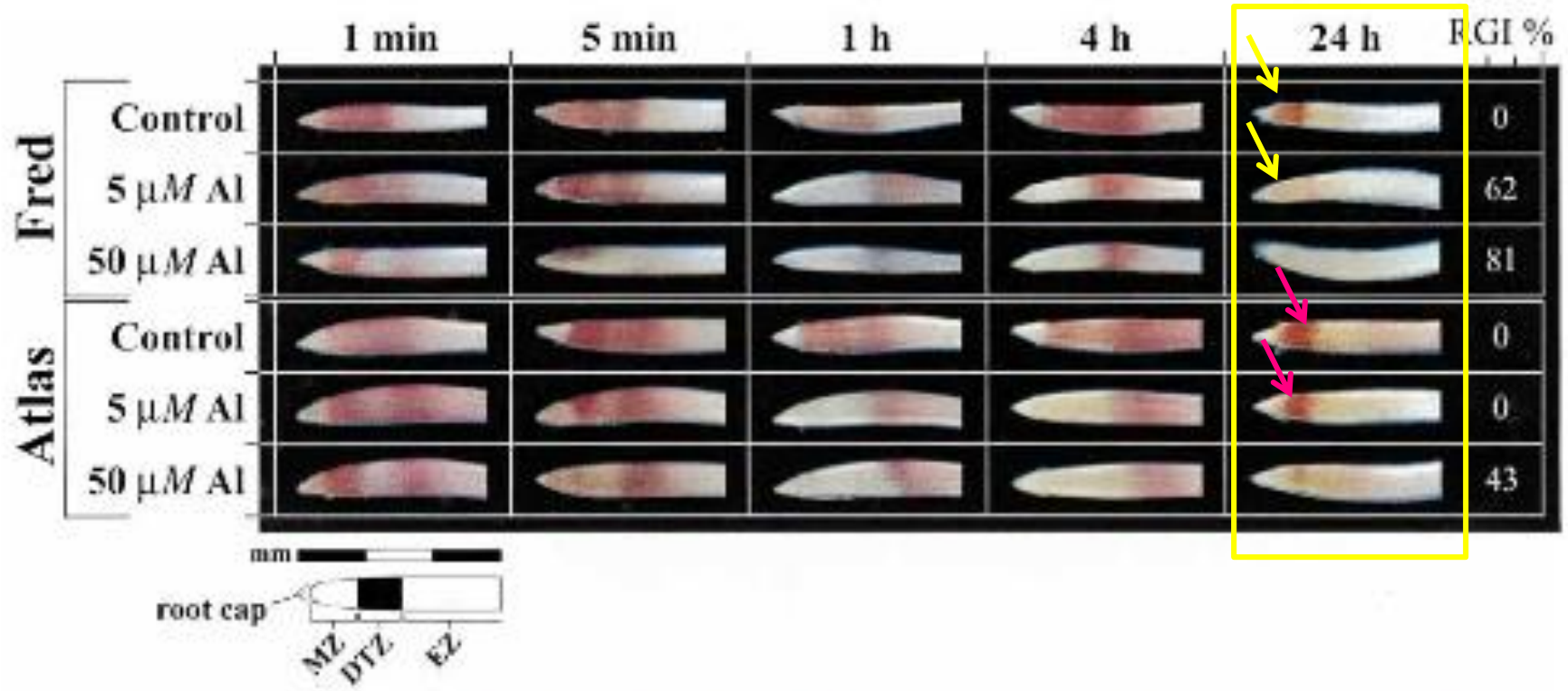


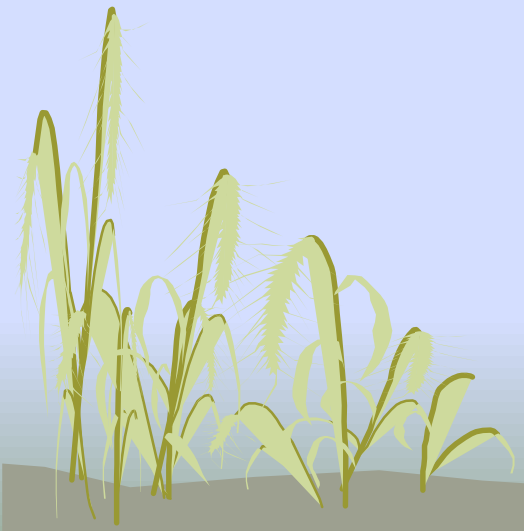
Fig. 2. Kinetics of NBT reduction activity in the Al-sensitive and the Al-tolerant cultivars exposed to various Al concentrations. Two wheat cultivars (Al sensitive, Fredrick: Fred; Al-tolerant, Atlas-66: Atlas), were exposed to the Al control solution (1 m*M* $CaCl_2$, pH4.15) or to the same solution 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

Cytological marker

- Markers that are related to variation in chromosome number, shape, size and banding pattern



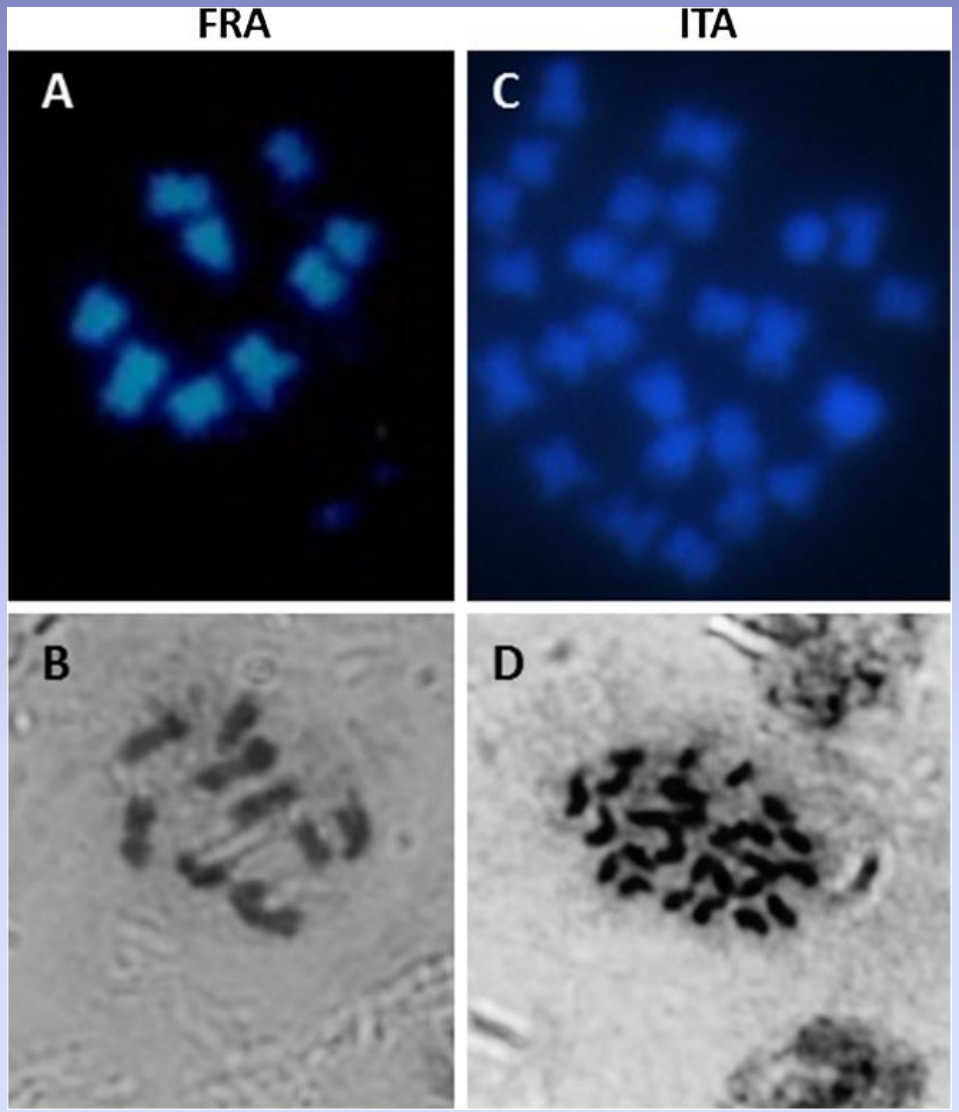


Figure 1 Determination of chromosome number by DAPI (A, C) and hematoxylin (B, D) staining of chromosomes showing counts of 9 in the haploid plants from France (FRA) and 27 in the tri-haploid plant from Italy (ITA). Chromosome count for the plant from Spain (ESP) was described in Aleza *et al.* [22].

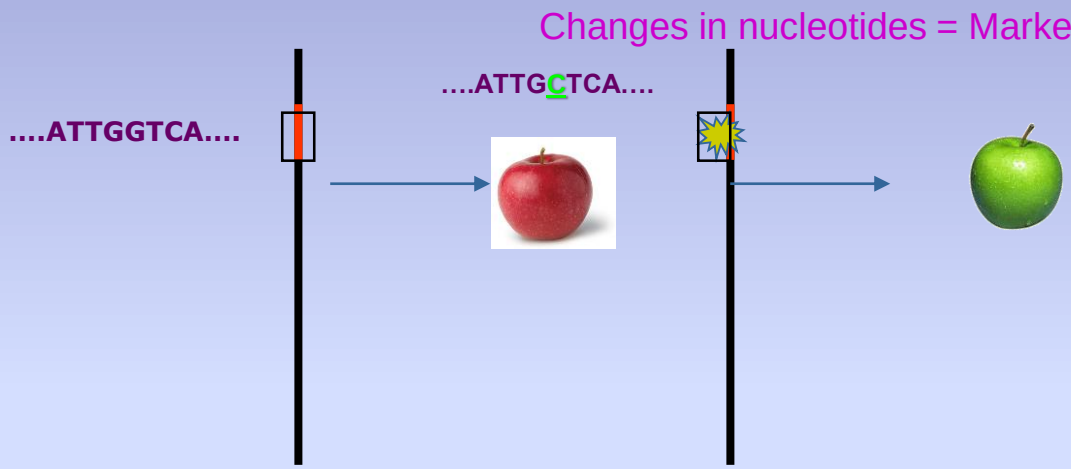
Germana *et al. BMC Plant Biology* 2013, **13**:129
http://www.biomedcentral.com/1471-2229/13/129

Molecular marker

- A molecule contained within a sample taken from an organism (biological markers) or other matter. It can be used to reveal certain characteristics about the respective source.

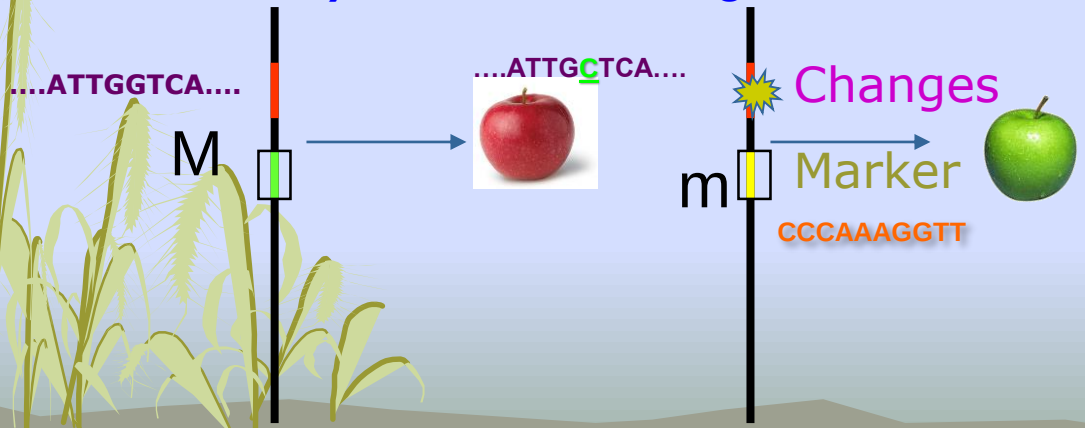
Two basic types of DNA-markers

- **DNA marker in coding region that cause the phenotypic change**



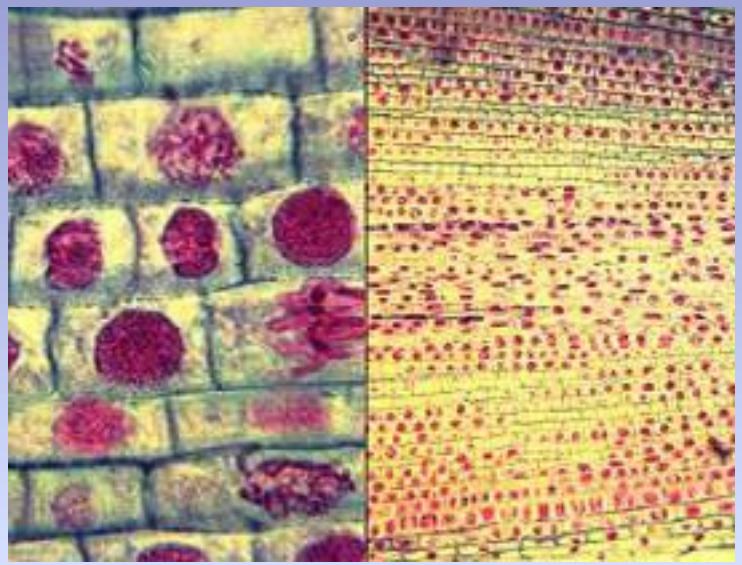
- The most useful
-Difficult to find

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

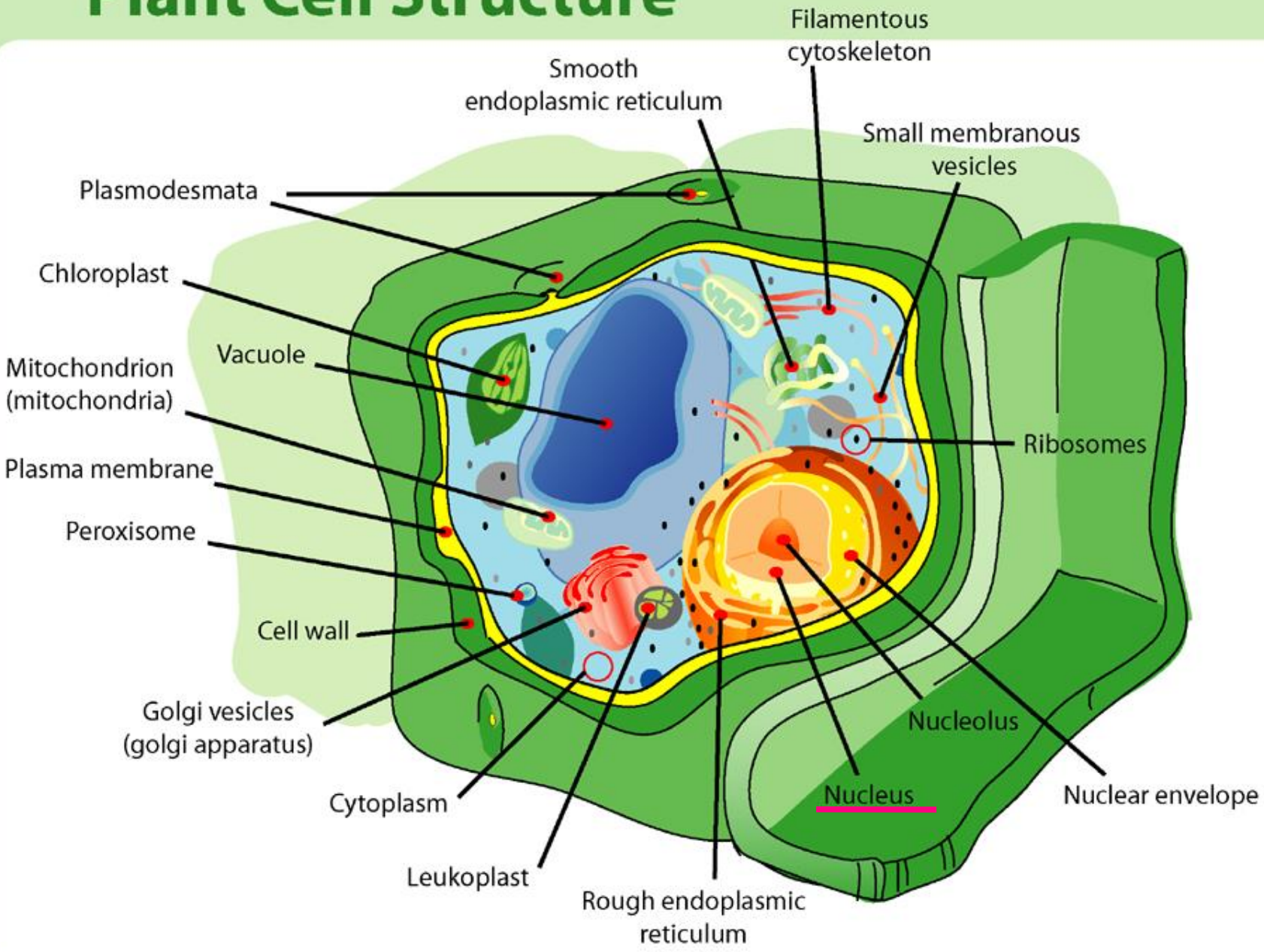


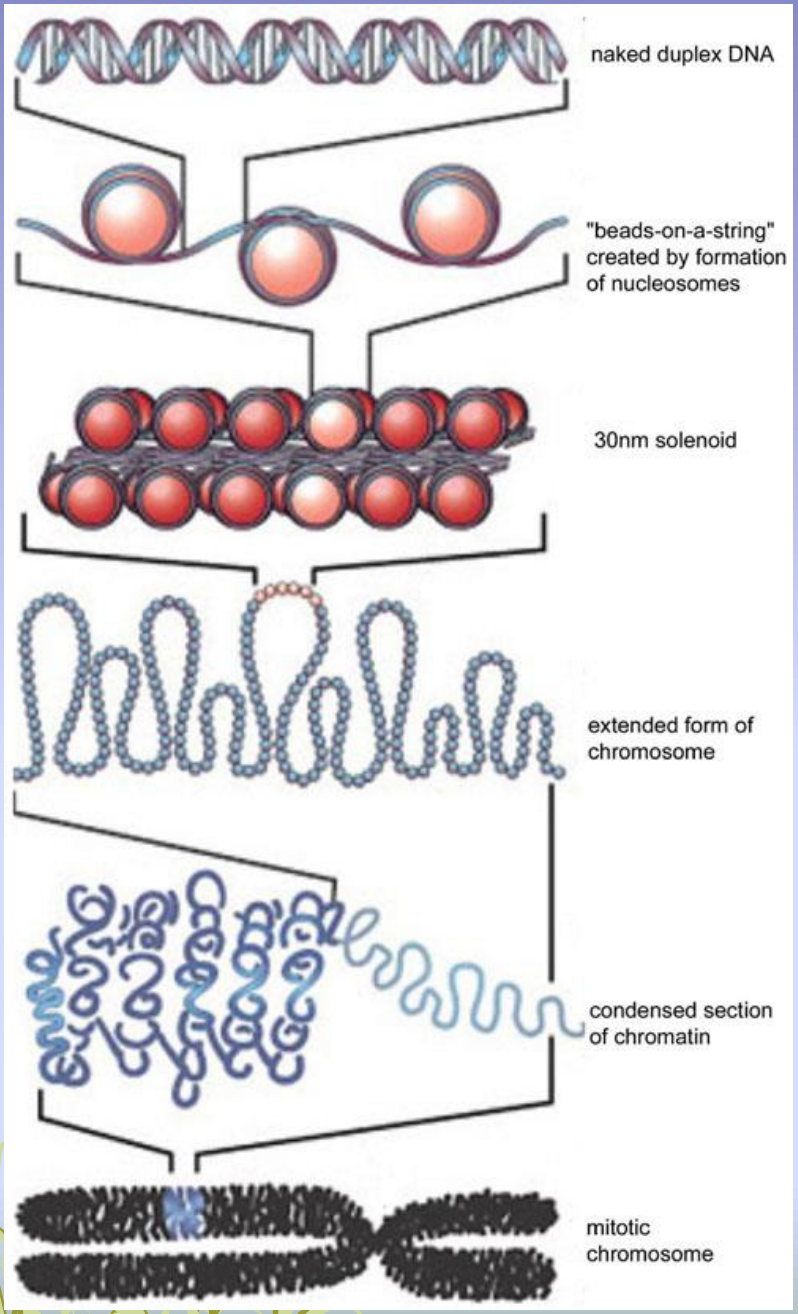
- Easier to find
- Frequently used

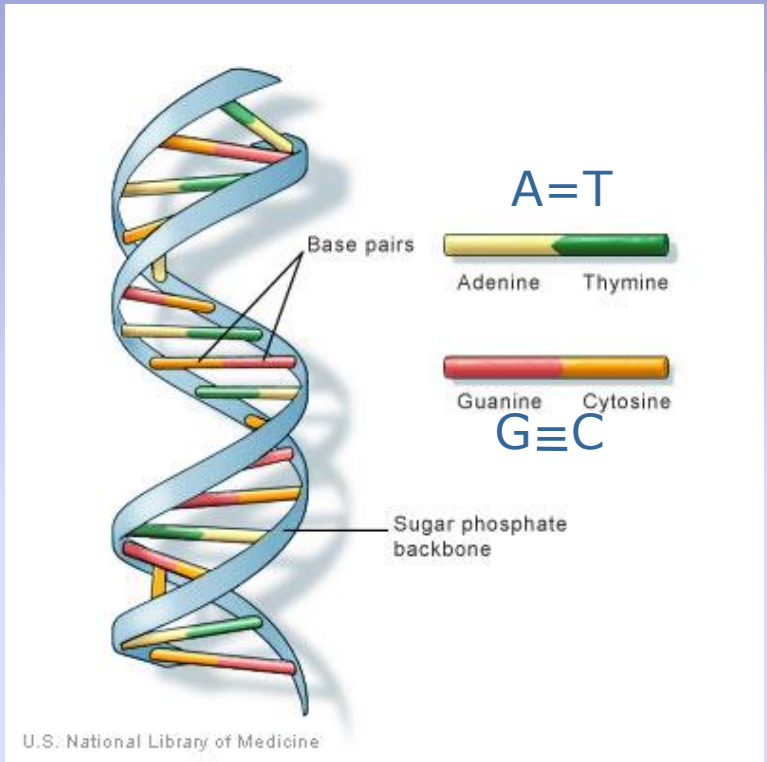
Plant chromosome



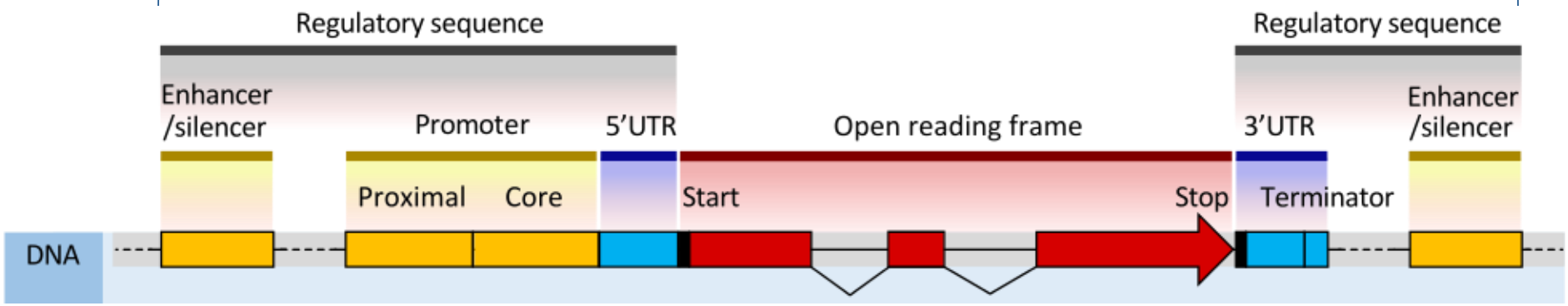
Plant Cell Structure

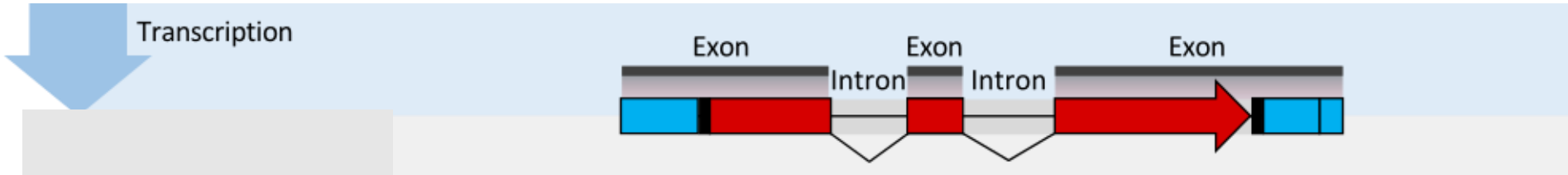


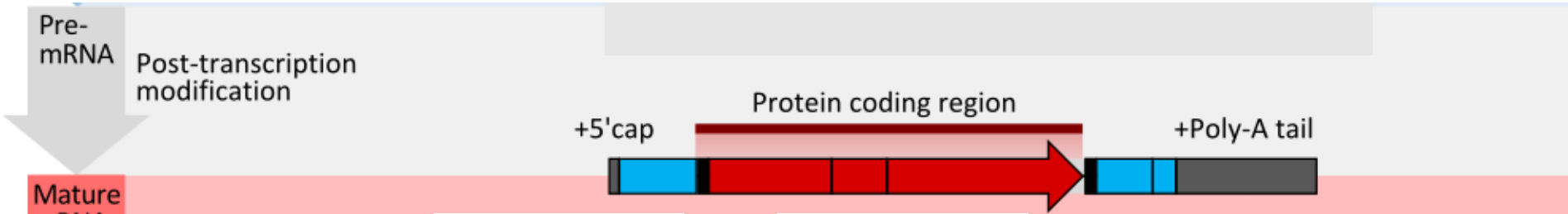

naked duplex DNA
"beads-on-a-string" created by formation of nucleosomes
30nm solenoid
extended form of chromosome
condensed section of chromatin
mitotic chromosome

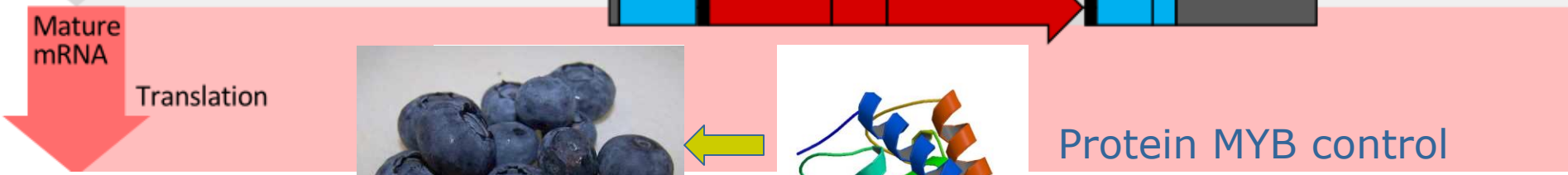

A=T
Base pairs
Adenine
Thymine
Guanine
Cytosine
G≡C
Sugar phosphate backbone
U.S. National Library of Medicine

Gene structure



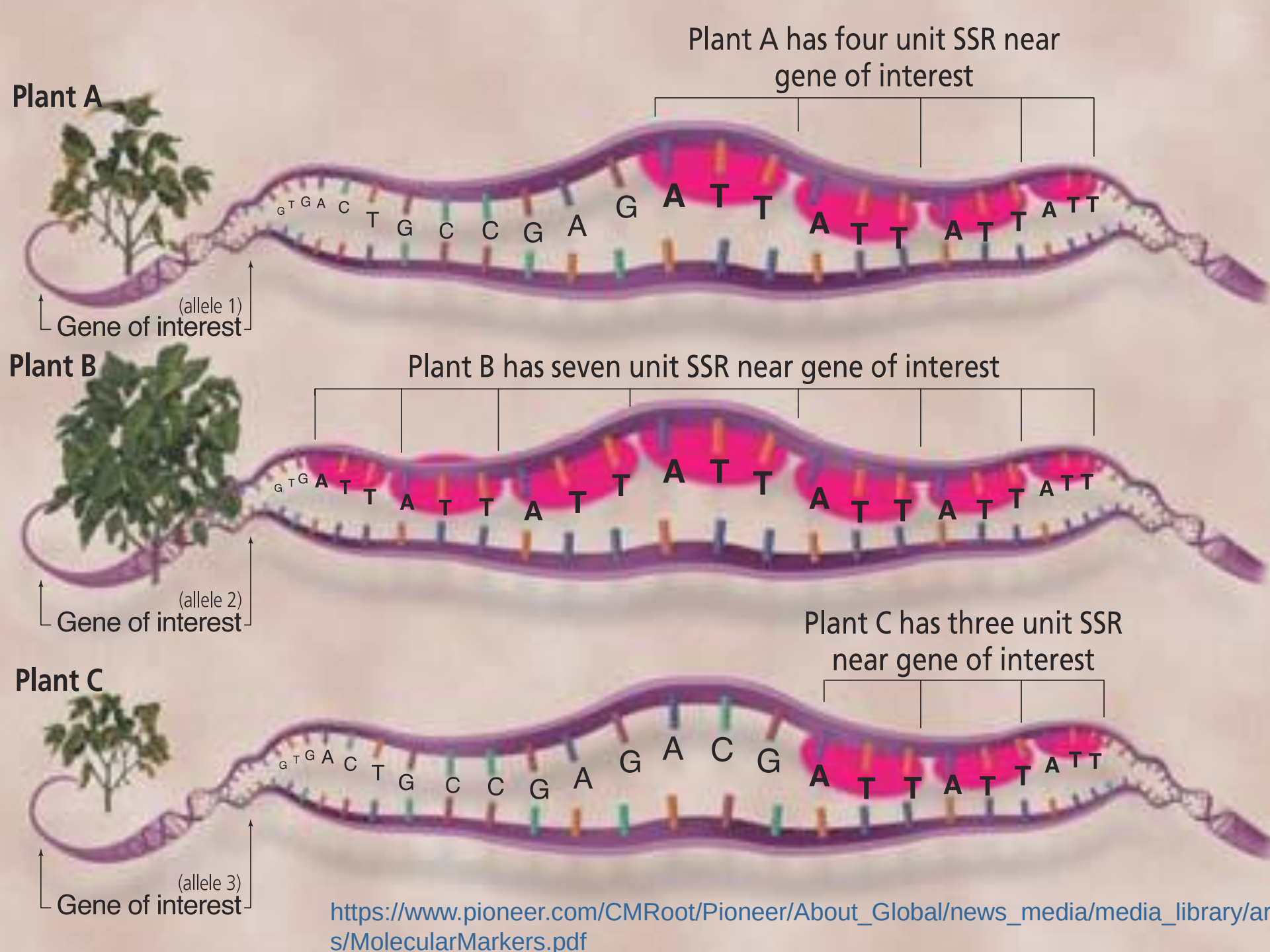






Protein MYB control the production of anthocyanin

WIKIMEDIA.ORG



https://www.pioneer.com/CMRoot/Pioneer/About_Global/news_media/media_library/ar
s/MolecularMarkers.pdf

Advantages of MAS

- **Simpler method compared to phenotypic screening**
  - Especially for traits with laborious screening
  - May save time and resources
- **Selection at seedling stage**
  - Important for traits such as grain quality
  - Can select before transplanting
- **Increased reliability**
  - No environmental effects
  - Can discriminate between homozygotes and heterozygotes and select single plants

Source: IRRI

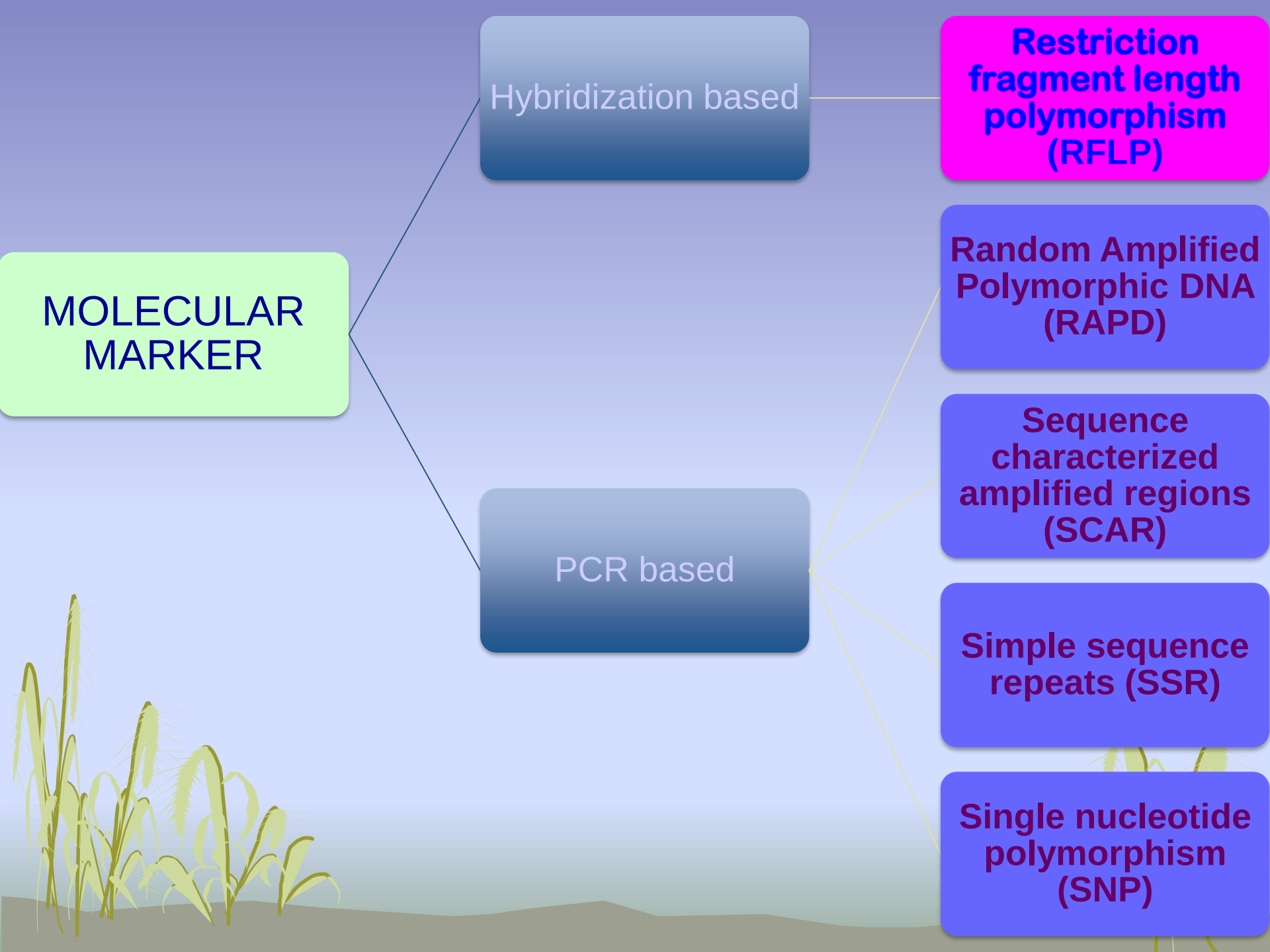
Potential benefits from MAS

- **More accurate and efficient selection of specific genotypes**
  - May lead to accelerated variety development
- **More efficient use of resources**
  - Especially field trials

Crossing house

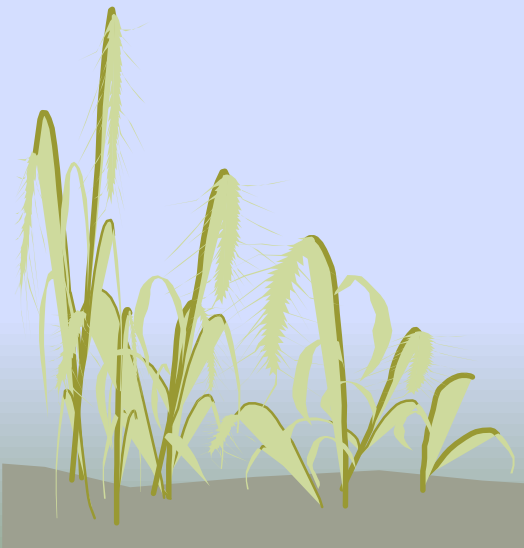
Backcross nursery

Source: IRRI

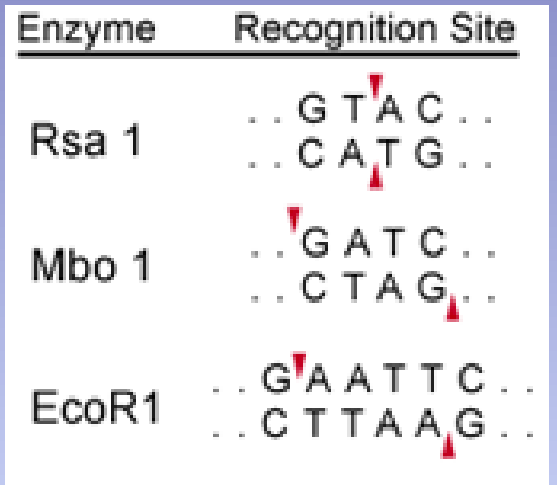

MOLECULAR MARKER
Hybridization based
Restriction fragment length polymorphism (RFLP)
PCR based
Random Amplified Polymorphic DNA (RAPD)
Sequence characterized amplified regions (SCAR)
Simple sequence repeats (SSR)
Single nucleotide polymorphism (SNP)

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.



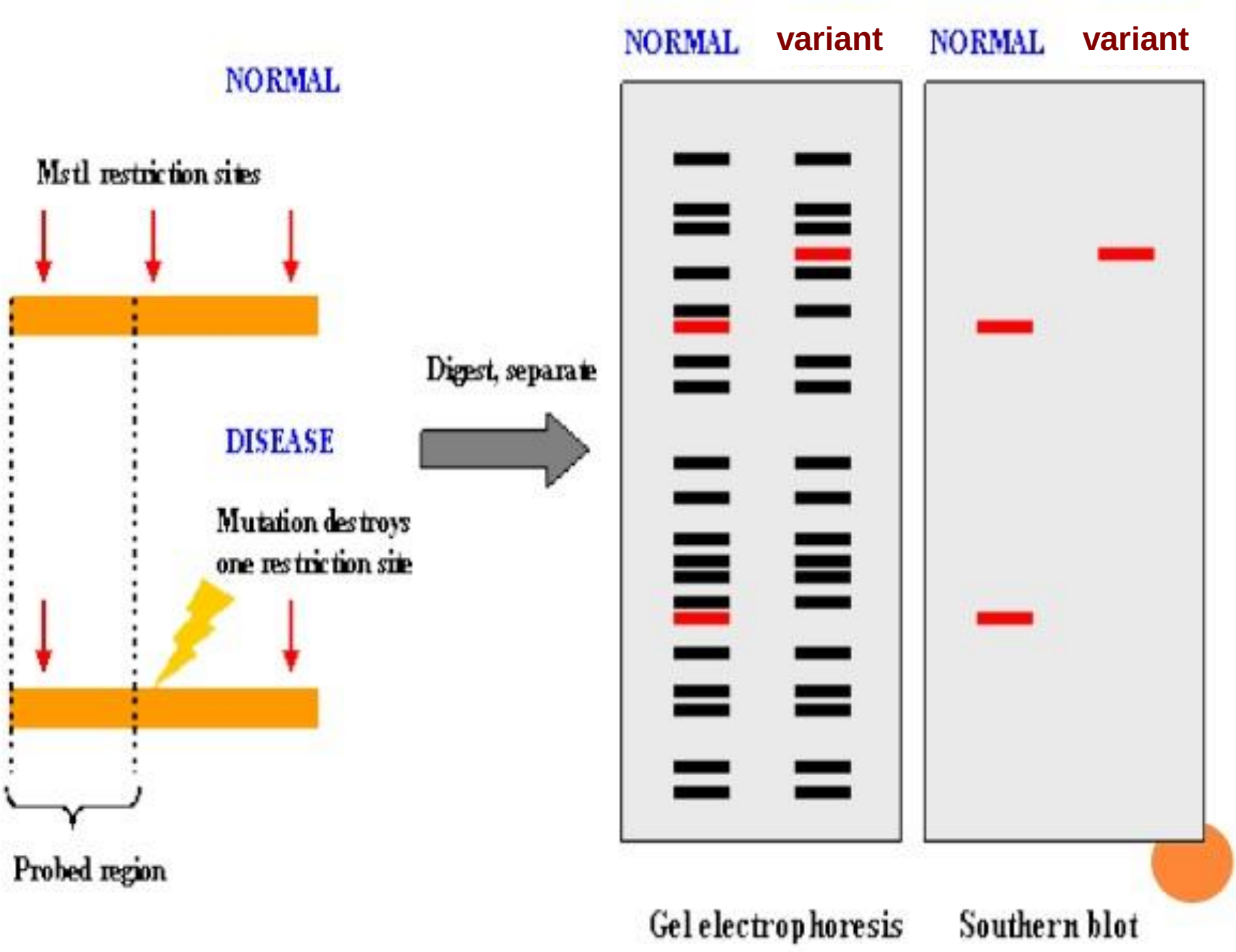
Restriction enzymes are proteins isolated from bacteria that recognize specific short sequences of DNA and cut the DNA at those sites. The normal function of these enzymes in bacteria is to protect the organism by attacking foreign DNA, such as viruses.



RsaI from Rhodopseudomonas sphaeroides (S. Kaplan)

MboI from *Moraxella bovis*

EcoRI from *Escherichia coli*


NORMAL
MstI restriction sites
DISEASE
Mutation destroys
one restriction site
Probed region
Digest, separate
NORMAL
variant
NORMAL
variant
Gel electrophoresis
Southern blot

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

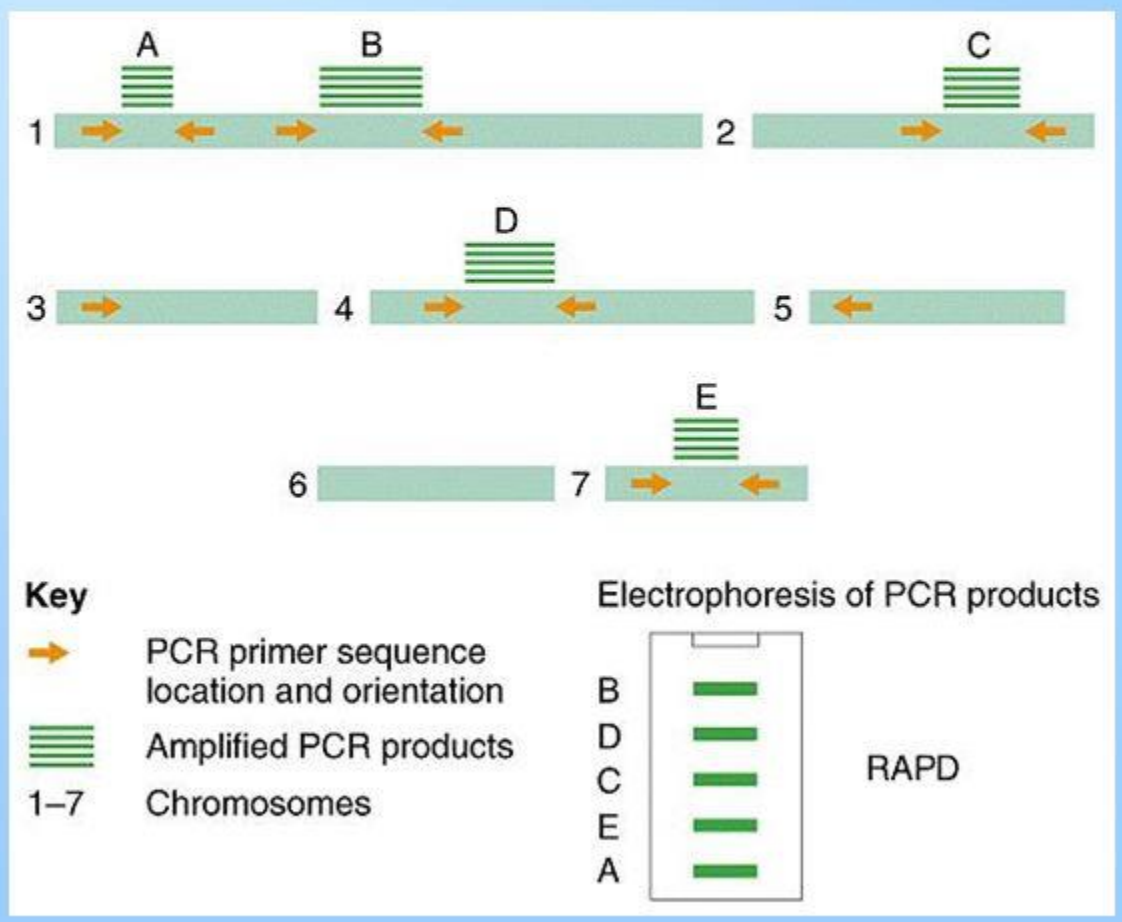
ORIGINAL PAPER

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

RAPD - Random Amplified Polymorphic DNA

- DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals
- Can be used with DNA of unknown sequence

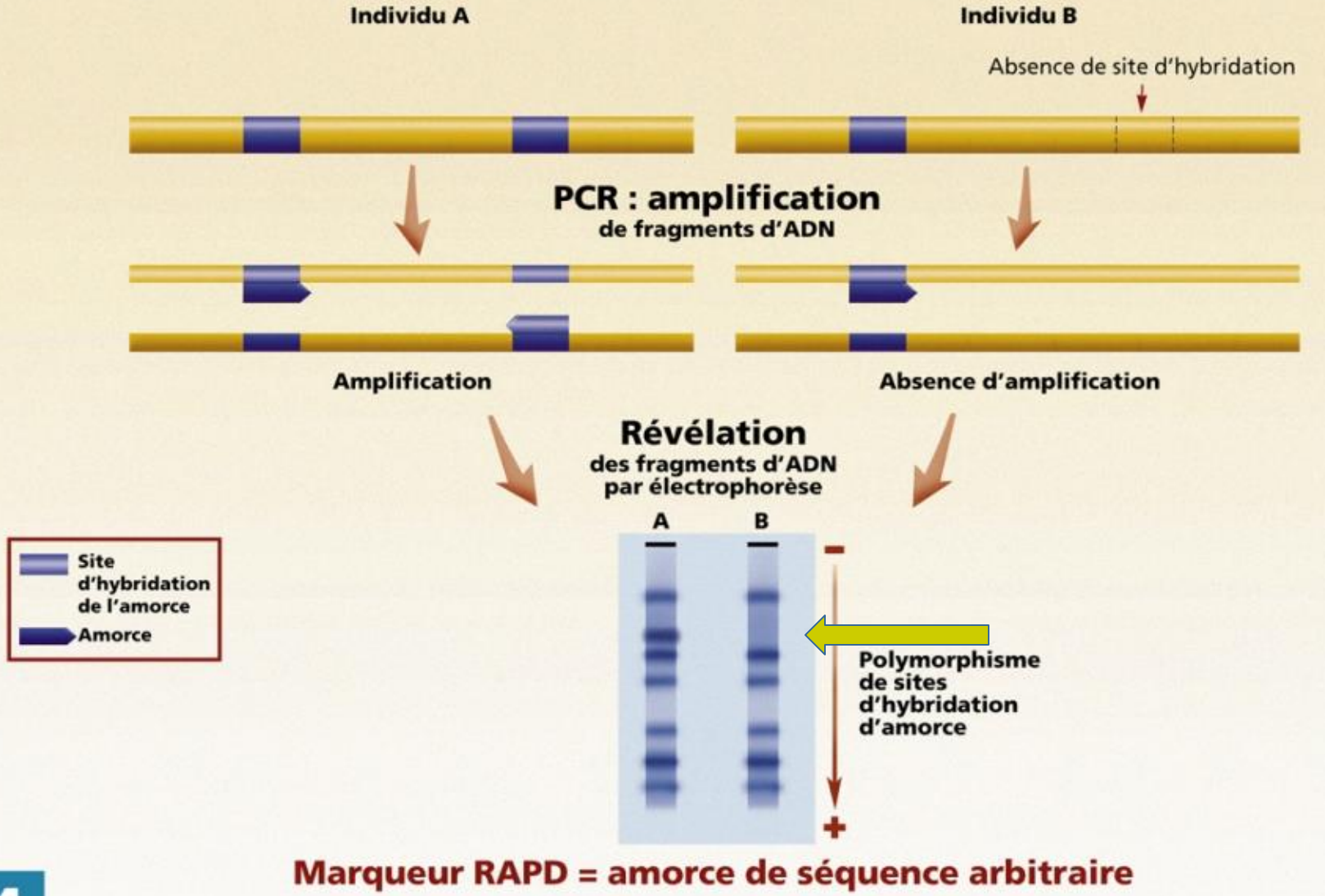
RAPD: randomly amplified polymorphic DNA



Size sorted

Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC & Gelbart WM (1996). An Introduction to Genetic Analysis, 6th edn. W.H. Freeman and Co., NY.

Les marqueurs RAPD



Marqueur RAPD = amorce de séquence arbitraire

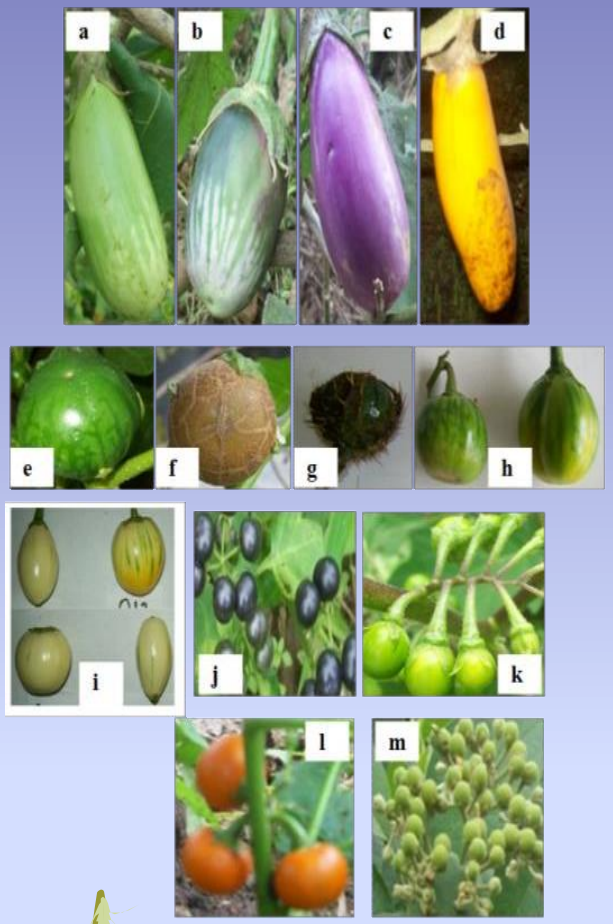


Figure 1. Variability in fruit colour and shape of some eggplant samples studied. Legend: (a - d) S. melongena; (e and f) S. macrocarpon; (g) S. dasyphyllum; (h and i) S. gilo; (j) S. scabrum; (k) S. incanum; (l) S. aethiopicum; (m) S. erianthum.

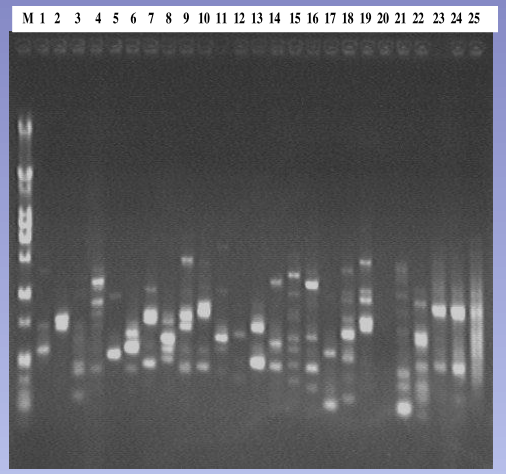


Figure 2. RAPD profiles generated by primer B-18 for *Solanum* samples studied. Legend: M represents the 100 bp DNA Ladder which serves as the reference point; 1 to 25 corresponds to bands produced by the amplified DNA from the 25 samples.

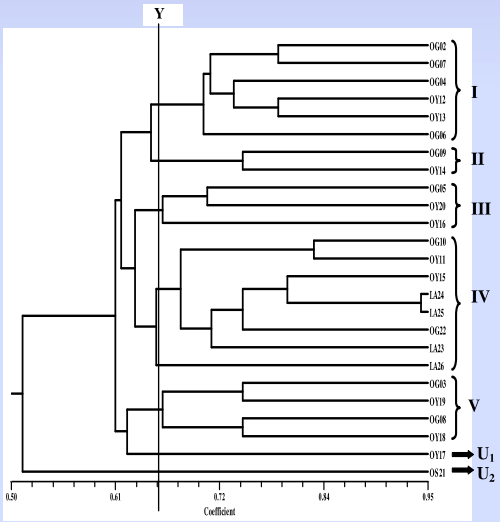


Figure 3. A UPGMA dendrogram showing genetic relationship among accessions of eggplants studied. Legend: Y represents truncated line at a co-efficient of similarity 0.65; I to V represent the five clusters that were distinguishable from the dendrogram while U_1 and U_2 represent ungrouped samples at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

academicJournals

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DOI: 10.5897/IJGMB2013.0089
ISSN 2006-9863 © 2014 Academic Journals
http://www.academicjournals.org/IJGMB

International Journal of Genetics and Molecular Biology

Full Length Research Paper

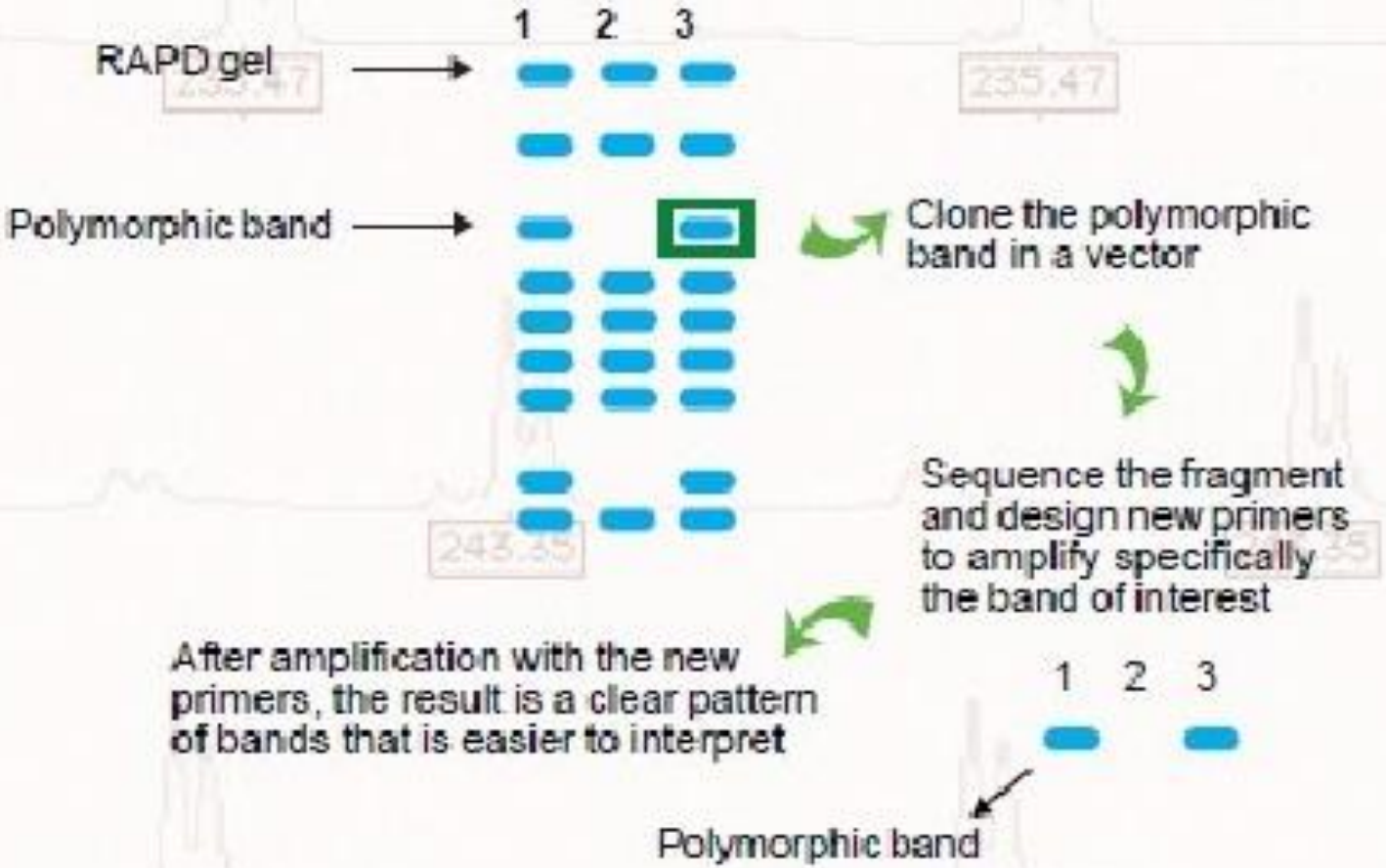
Partitioning and distribution of random amplified polymorphic DNA (RAPD) variation among eggplant Solanum L. in Southwest Nigeria

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

SCAR - *Sequence characterized amplified regions*

- SCARs are DNA fragments amplified by the Polymerase Chain Reaction (PCR) using specific 15-30 bp primers, designed from nucleotide sequences established in cloned RAPD (Random Amplified Polymorphic DNA) fragments linked to a trait of interest.

Diagram of the SCAR procedure



Indian J. Genet., 76(1): 116-118 (2016)
DOI: 10.5958/0975-6906.2016.00018.3

Short Communication

A molecular marker linked to the male gender of *Actinidia arguta* Siebold & Zucci

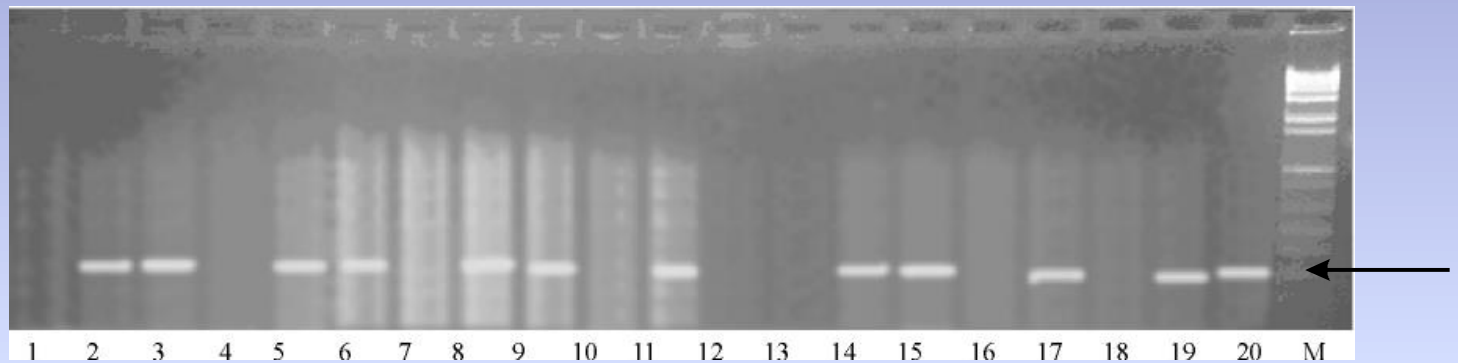
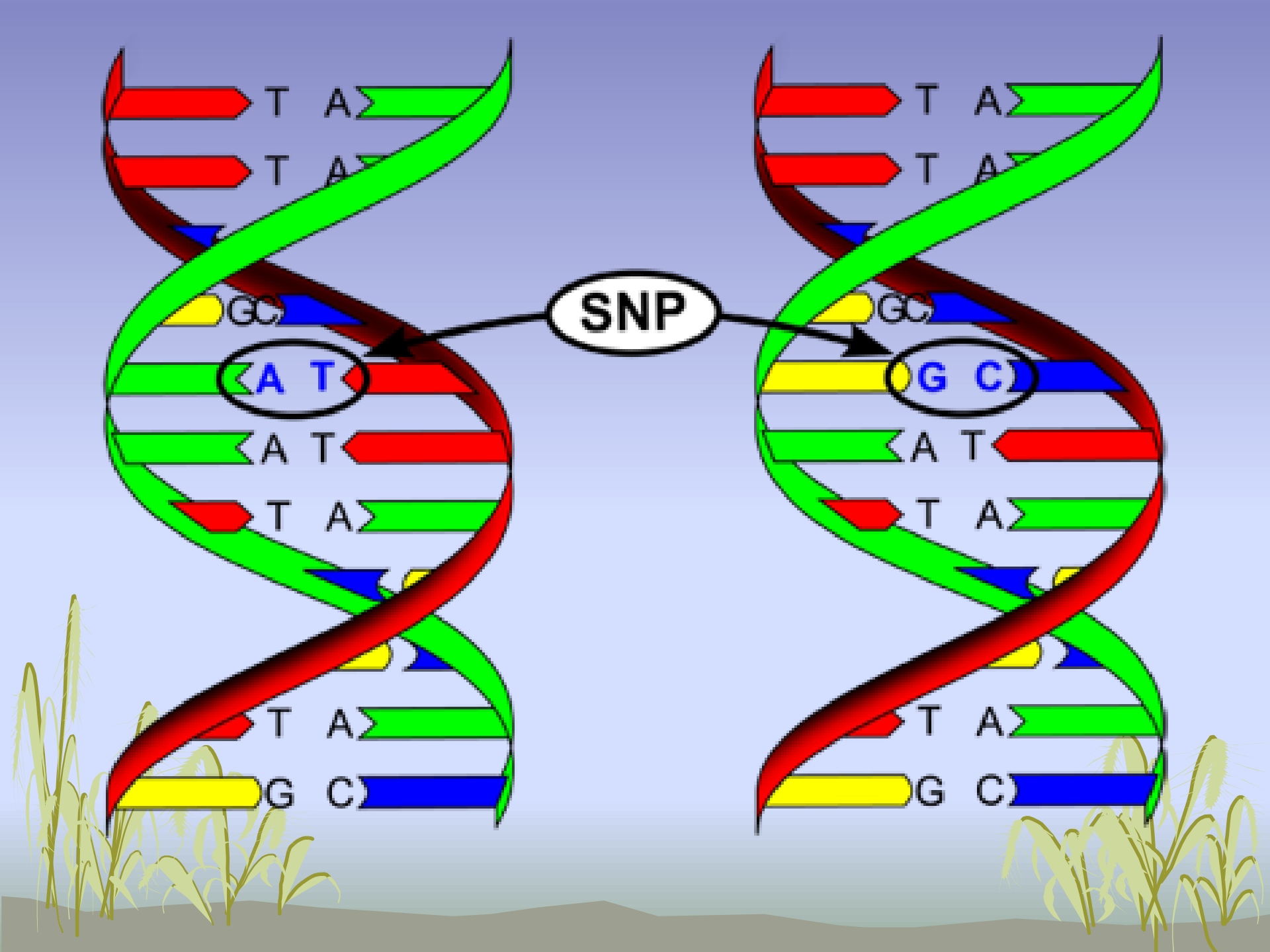


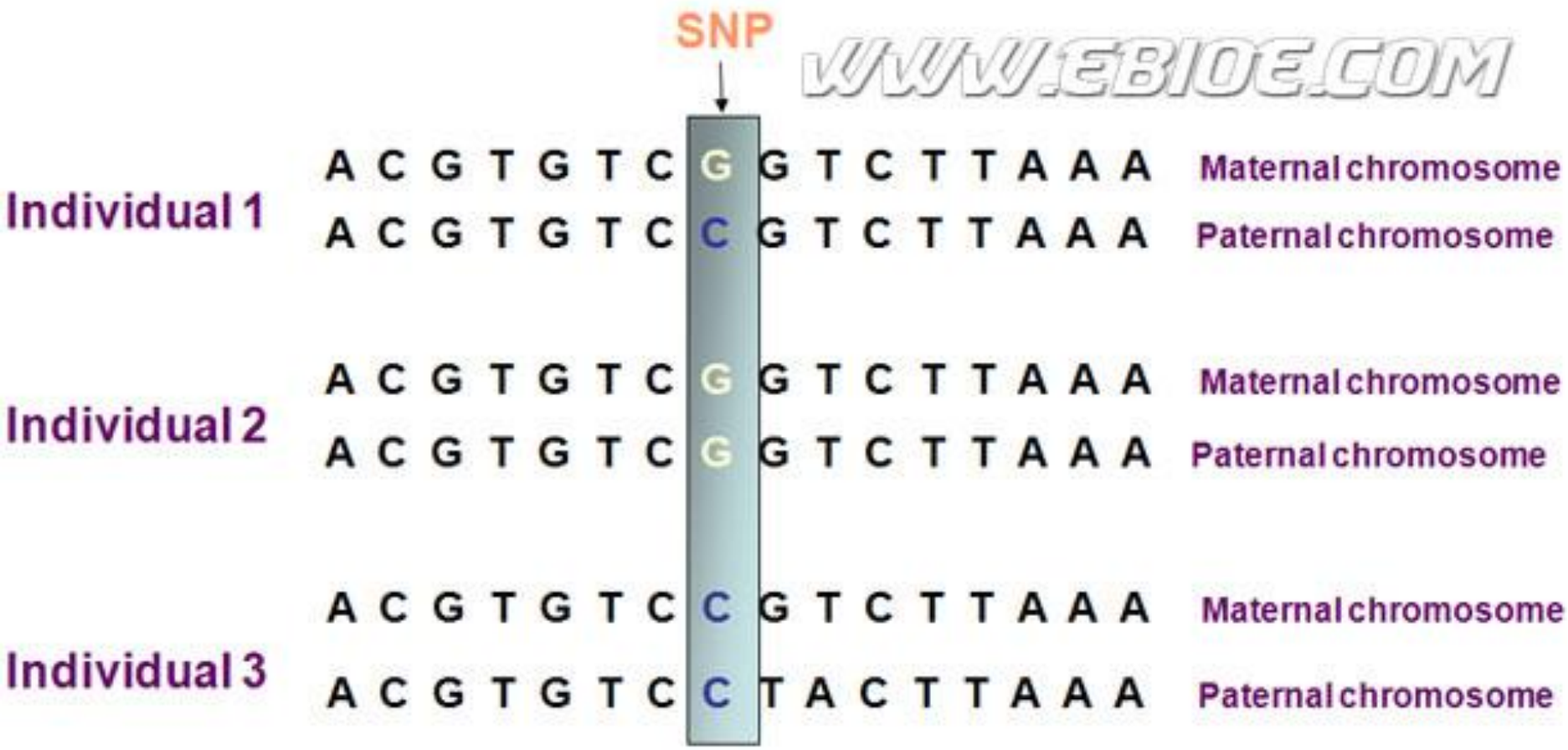
Fig. 2. Portion of amplification products of SCAR in hardy kiwi F_1 plants. 1-20 = different accessions of F_1 generation

SCAR marker

SNP - Single nucleotide polymorphism

- a DNA sequence variation occurring when a single nucleotide adenine (A), thymine (T), cytosine (C), or guanine (G]) in the genome differs between members of a species or paired chromosomes in an individual.
- Single nucleotides may be changed (substitution), removed (deletions) or added (insertion) to a polynucleotide sequence
- SNPs may fall within coding sequences of genes, non-coding regions of genes, or in the intergenic regions between genes


T A
T A
G C
A T
A T
T A
T A
G C
SNP
T A
T A
G C
G C
T A
T A
G C



The position of the SNP is indicated by the box. Individual 1 is *heterozygous*, while individuals 2 and 3 are *homozygous*.

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

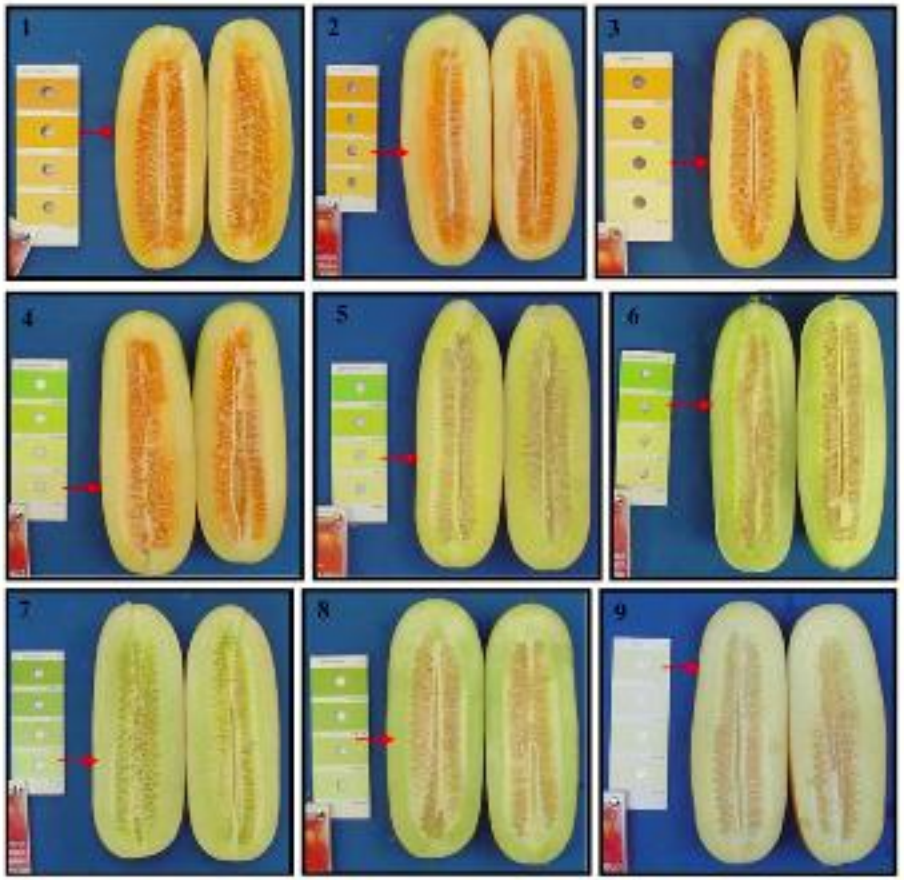
REVIEW

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]

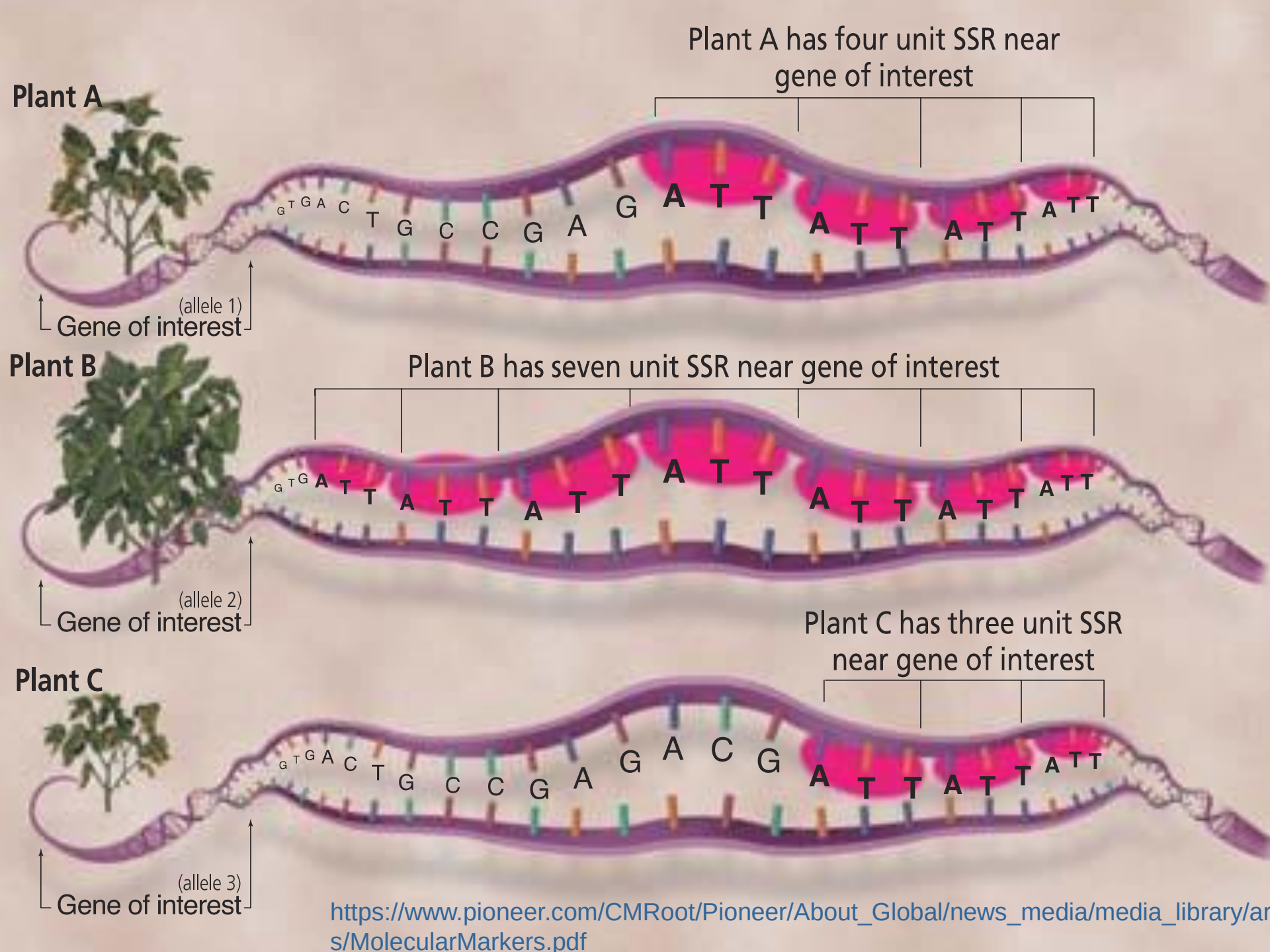
Theor Appl Genet (2014) 127:2051–2064
DOI 10.1007/s00122-014-2360-5

ORIGINAL PAPER

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

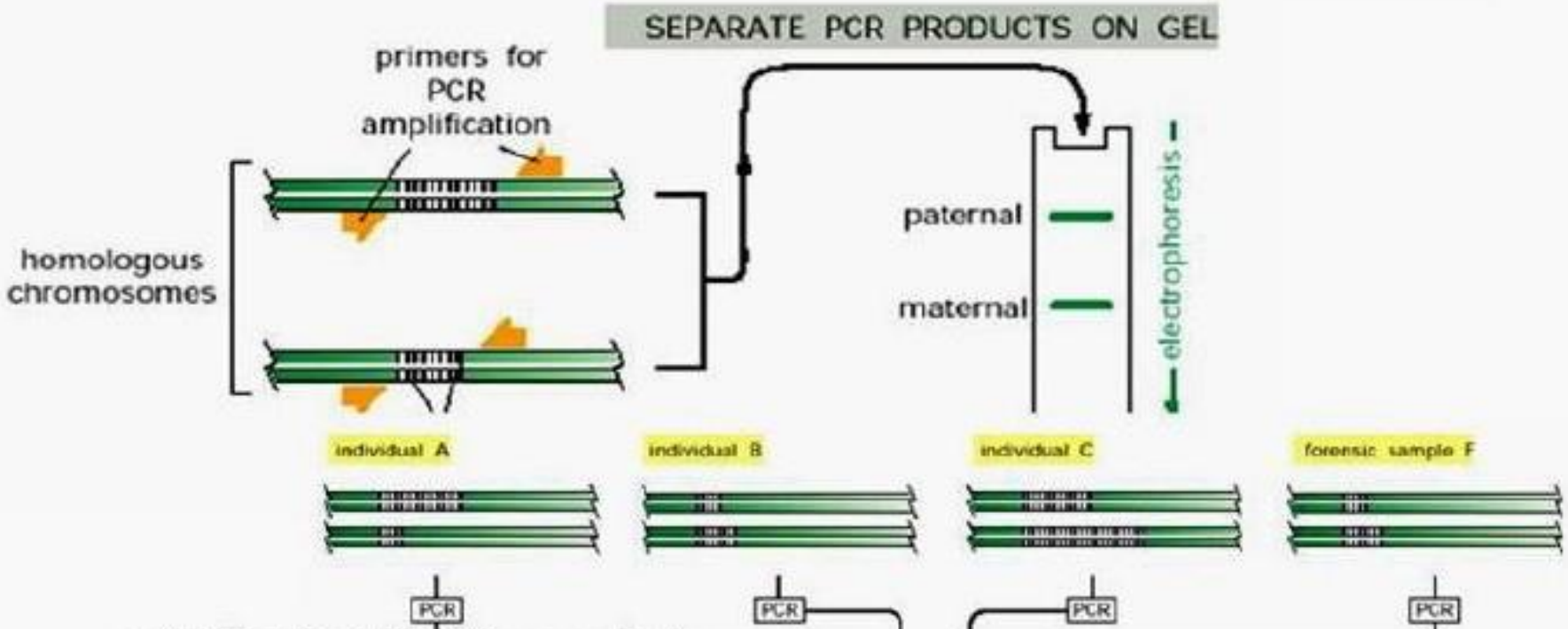
SSR - Simple sequence repeats

- DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals
- Can be used with DNA of unknown sequence

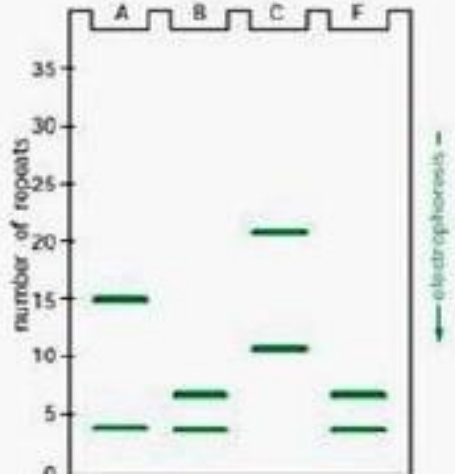


https://www.pioneer.com/CMRoot/Pioneer/About_Global/news_media/media_library/ar
s/MolecularMarkers.pdf

Microsatellites [STRs/SSRs]



- Applications in linkage analysis, genotyping, genetic identity
- **Population Genetics & Conservation**
- Becoming more popular in agricultural field

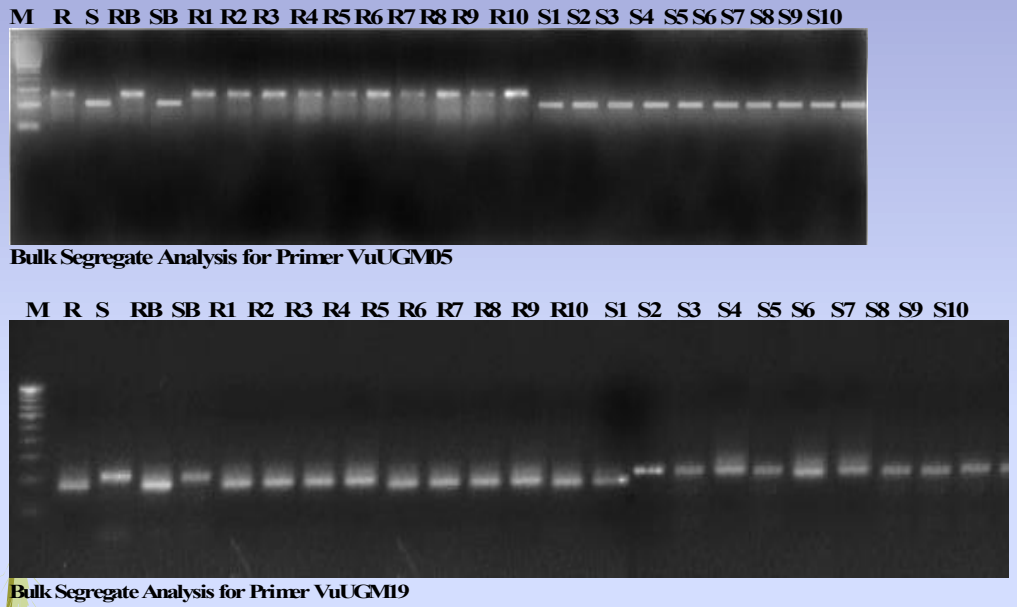


http://image.slidesharecdn.com/microsatellite-120515062853-phpapp01/95/microsatellite-9-728.jpg?cb=1337063766

Legume Research, 39 (1) 2016: 39-42
Print ISSN:0250-5371 / Online ISSN:0976-0571

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.legumeresearch.in

Identification of SSR marker associated with rust resistance in cowpea (*Vigna unguiculata* L.) using bulk segregant analysis



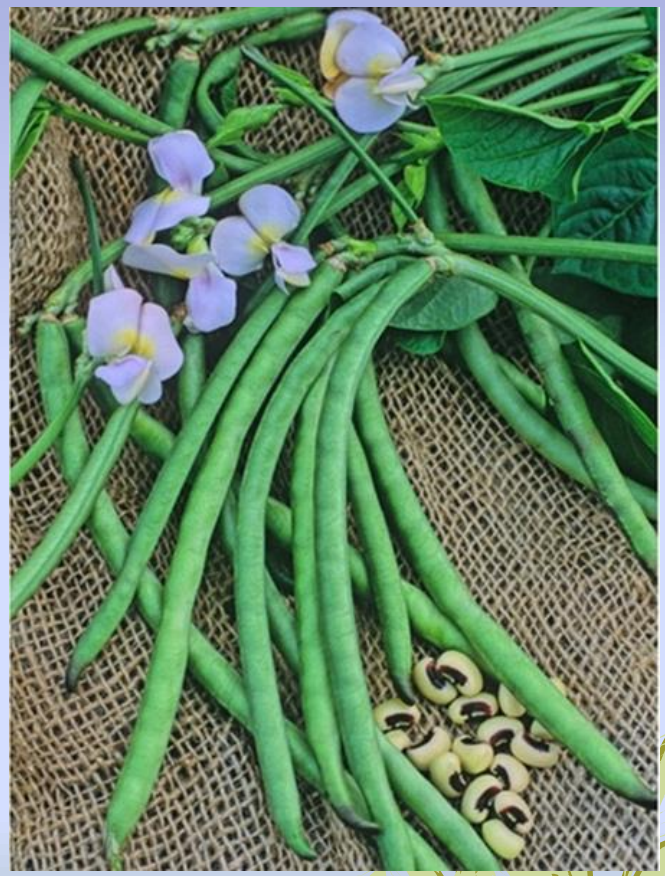


FIG 2: SSR markers VuUGM05 and VuUGM19 revealed clear cut difference in both parents as well as in two bulks.

M-Marker
R-Resistant
S-Susceptible
RB-Resistant Bulk
SB-Susceptible bulk
R_1-R_{10}-Resistant Individual Plants
S_1-S_{10}-Susceptible Individual Plants

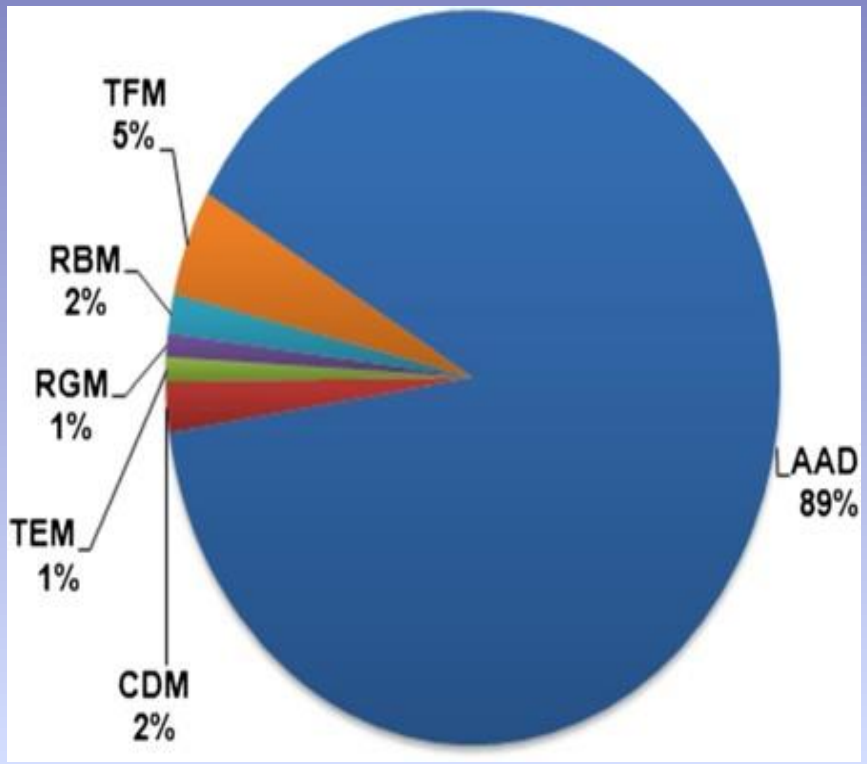


Figure 1 Percentages of studies utilizing different types of molecular markers. The chart is based on an informal literature search performed with Google Scholar on 22.08.2012 resulting in 1032570 hits. Abbreviations are according to acronyms found in the text: **AAD** – Arbitrarily amplified DNA markers, 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

Table 1 Summary table of marker systems and groups

Group	Marker system	Principle in a nutshell	References
(1) Conserved DNA and gene family based markers (CDMs)	(1.1) CDDP	Conserved plant genes are targeted with short universal or degenerate primers to reveal length polymorphism. Use of primer combinations is also possible.	Collard and Mackill [57]
	(1.2) PBA	Universal primers target the exon-intron junction sites of cytochrome (cyt) P450 mono-oxygenases. Polymorphism is revealed based on the random distribution of gene family members.	Yamanaka et al. [58]
	(1.3) TBP	Single degenerate primer pairs anneal to the conserved parts of the β-tubulin exons and amplify intercalated introns from different tubulin isotypes.	Bardini et al. [59]; Breviario et al. [60]; Galasso et al. [61]
	(1.4) ITP	Intron regions of choice are amplified by exon flanking primers revealing polymorphism.	Weining and Langridge [62]
(2) Transposable element based markers (TEMs)	(2.1) IRAP	Amplification of internal sequences between two retrotransposon repeats with primers annealing to LTR motifs.	Kalendar et al.[63]
	(2.2) REMAP	An LTR specific primer and an ISSR primer are used to detect polymorphism.	Kalendar et al. [63]
	(2.3) ISAP	Primers designed in various positions within SINE elements are used to amplify adjacent genomic regions.	Seibt et al. [64]
	(2.4) iPBS	Primers anneal to PBS regions of head-to-head oriented LTR retrotransposons. The amplified products contain LTR regions and intervening genomic regions.	Kalendar et al. [65]
	(2.5) SSAP	DNA is digested with restriction enzymes. Adapters are ligated to restriction sites, and amplification is performed with LTR specific and adapter specific primers containing selective nucleotides.	Waugh et al. [66]

(3) Resistance-gene based markers (RGMs)	(3.1) RGAP	Resistance-gene based analogue fingerprints are generated with degenerate specific primers or primer pairs, designed to match conserved regions of R-genes.	Leister et al. [67]
	(3.2) NBS-profiling	Genomic DNA is digested with restriction enzymes after the ligation of adapters. Specific fingerprints are generated from resistance gene regions with adapter specific and R-gene specific primers.	Linden et al. [68]
(4) RNA-based markers (RBMs)	(4.1) iSNAP	Primers are designed from small RNAs and flanking regions to generate polymorphic banding patterns.	Gui et al. [69]
	(4.2) cDNA-AFLP	An AFLP analysis is carried out using cDNA as a starting pool, with several modifications existing for fine-tuning.	Bachem et al. [70]
	(4.3) cDNA-RFLP	cDNA is used for probes in RFLP analysis.	Bryan et al. [71]
	(4.4) EST-SSR	EST databases are mined *in silico* to locate SSRs and design primers to match genetic microsatellites.	Kantety et al. [72]
(5) Targeted fingerprinting markers (TFMs)	(5.1) DALP	The common M13 sequencing primer is paired with a forward primer containing the –40 USP core and 3′ selective nucleotides to generate fingerprints.	Desmarais et al. [73]
	(5.2) PAAP	Degenerate regions annealing to plant promoter regions are added to short oligonucleotides to detect polymorphism.	Pang et al. [74]
	(5.3) SRAP	Primers contain a random 5′ filter, a core sequence (AATT or CCGG) and three variable nucleotides at their 3′. Amplification follows a two step procedure where first mismatches are allowed at a lower temperature to generate a starting pool for subsequent higher temperature amplification.	Li and Quiros [75]
	(5.4) TRAP	An arbitrary SRAP primer is paired with a fixed primer designed from ESTs.	Hu and Vick [76]
	(5.5) CoRAP	Arbitrary primers are designed from ESTs as in TRAP, but the fixed primer contains a different core (CACGC), as in SRAP. This sequence is often found in plant introns.	Wang et al. [77]
	(5.6) SCoT	ATG start codons are incorporated into random primers to generate polymorphic fragments from the genome. Primers can be used alone or in combination.	Collard and Mackill [78]

Table 2 Comparison of various aspects of gene-targeted and functional marker techniques

	Conserved DNA and gene family based markers (CDMs)				Transposable element based markers (TEMs)			
	CDDP	PBA	TBP	ITP	IRAP/REMAP	ISAP	iPBS	SSAP
Abundance	Medium, may depend on targeted genes	High	Medium	Low, may depend on targeted genes	High	High	High	High
Reproducibility	High	High	High	High	Medium	High	High	High
Polymorphism	Medium	High	High	Medium	Medium	Medium	High	High
Prior sequence information	Yes	No	No	Yes	Yes	Yes	No	Yes
Visualization	Agarose gel electrophoresis	Agarose gel electrophoresis	Agarose gel electrophoresis or silver stained PAGE	Agarose gel electrophoresis sometimes with high resolution	Agarose gel electrophoresis	Agarose gel electrophoresis	Agarose gel electrophoresis	Silver stained PAGE
Specificity	Not reported	High	High	High	High	High	High	High
Size of bands	200-1,500 bp	100-1,500 bp	500-2,000 bp	50-800 bp	100-5,000 bp (up to 10 kbp)	250-2,500 bp	100-5,000 bp	50-500 bp
Homoplasy	High	High	Low	Low	Medium	Not reported	Low	Low
Reaction artifacts								
i. Uniparental bands	Not reported	Not reported	No	No	No	Not reported	No	No
ii. Heteroduplexes	Not reported, but may occur	Not reported, but may occur	Not reported, but may occur	Yes	No	No	No	No
iii. Nested priming	Not reported	Not reported, but may occur	No	No	May occur	Not reported	Not reported	May occur
iv. Other	Amplicons may be generated from pseudogene loci				Inconsistencies in bands associated with TE activity	No	No	Inconsistencies in bands associated with TE activity

doi:10.3732/apps.1400017

TABLE 1. A summary of the attributes of different dominant molecular markers used in plant biology, and a relative comparison of their attributes.

Marker	PCR protocol	Genomic target	Relative cost	Dominance	Repeatability
RAPD	one-step	anonymous	low	dominant	moderate
ISSR	one-step	anonymous	low	dominant	high
AFLP	multistep	anonymous	moderate	mixed (low)	high
SRAP	one-step	coding	low	mixed (moderate)	high

Note: AFLP = amplified fragment length polymorphism; ISSR = inter-simple sequence repeat; RAPD = random-amplified polymorphic DNA; SRAP = sequence-related amplified polymorphism.

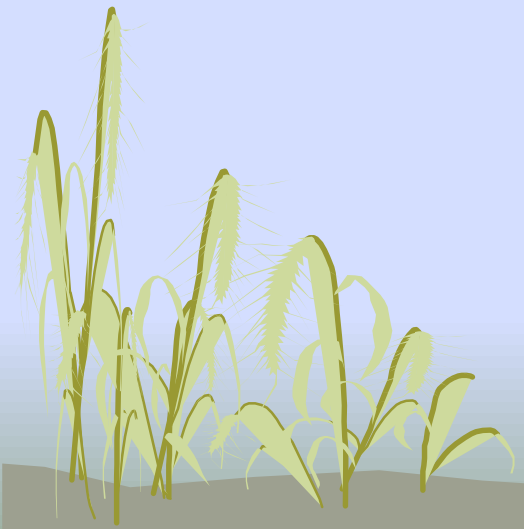
Application of markers in crop improvement

- 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

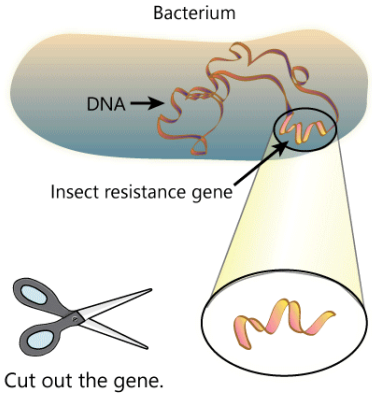
GENETIC ENGINEERING

Genetic engineering

- Addition of a foreign gene or genes or DNA to the genome of an organism. A gene holds information that will give the organism a trait.

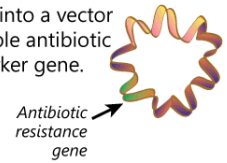


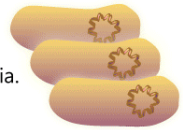
Creation of an Insect Resistant Tomato Plant



1. Cut out the gene.

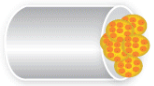
2. Insert gene into a vector with a selectable antibiotic resistance marker gene.





3. Copy vector in bacteria.

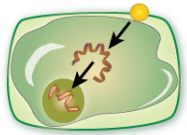
4. Coat tungsten or gold particles with DNA vectors.



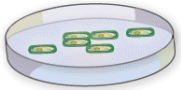
5. Load vector-coated particles onto teflon bullet.

6. Load bullet into gene gun.

7. Shooting the gene gun releases the particles at a high velocity penetrating the plant cells.

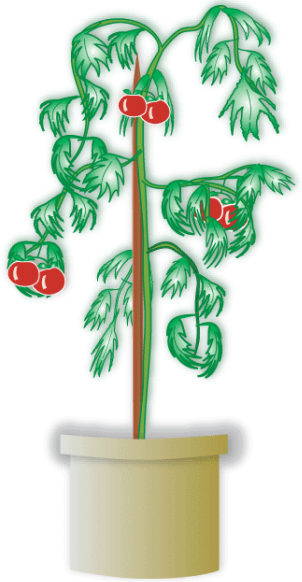


8. The vector enters the cell. The genes are incorporated into the plant genome.



9. The cells are plated on a selective antibiotic media. Only cells that have incorporated the vector will grow.

10. These cells are transferred to medium containing plant growth factors.



Insect resistant tomato plant

GM plant development

Choice of genes/proteins	Agronomic assessment	Characterization of gene product and comparative analysis	Postmarket assessment
• Source • Initial molecular characterization • History of safe use • Mode of action	• Greenhouse to field • Agronomic performance • Phenotypic screening of events • Event selection (<1%)	• Toxicity • Allergenicity • Nutrition • Compositional analysis • Environment • Further molecular characterization	• Postmarket surveillance • Supplemental food/feed studies, as needed

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

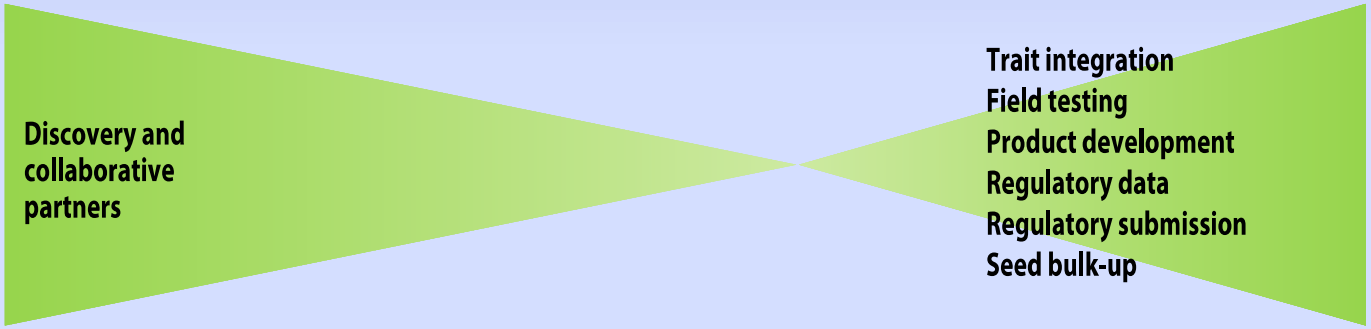
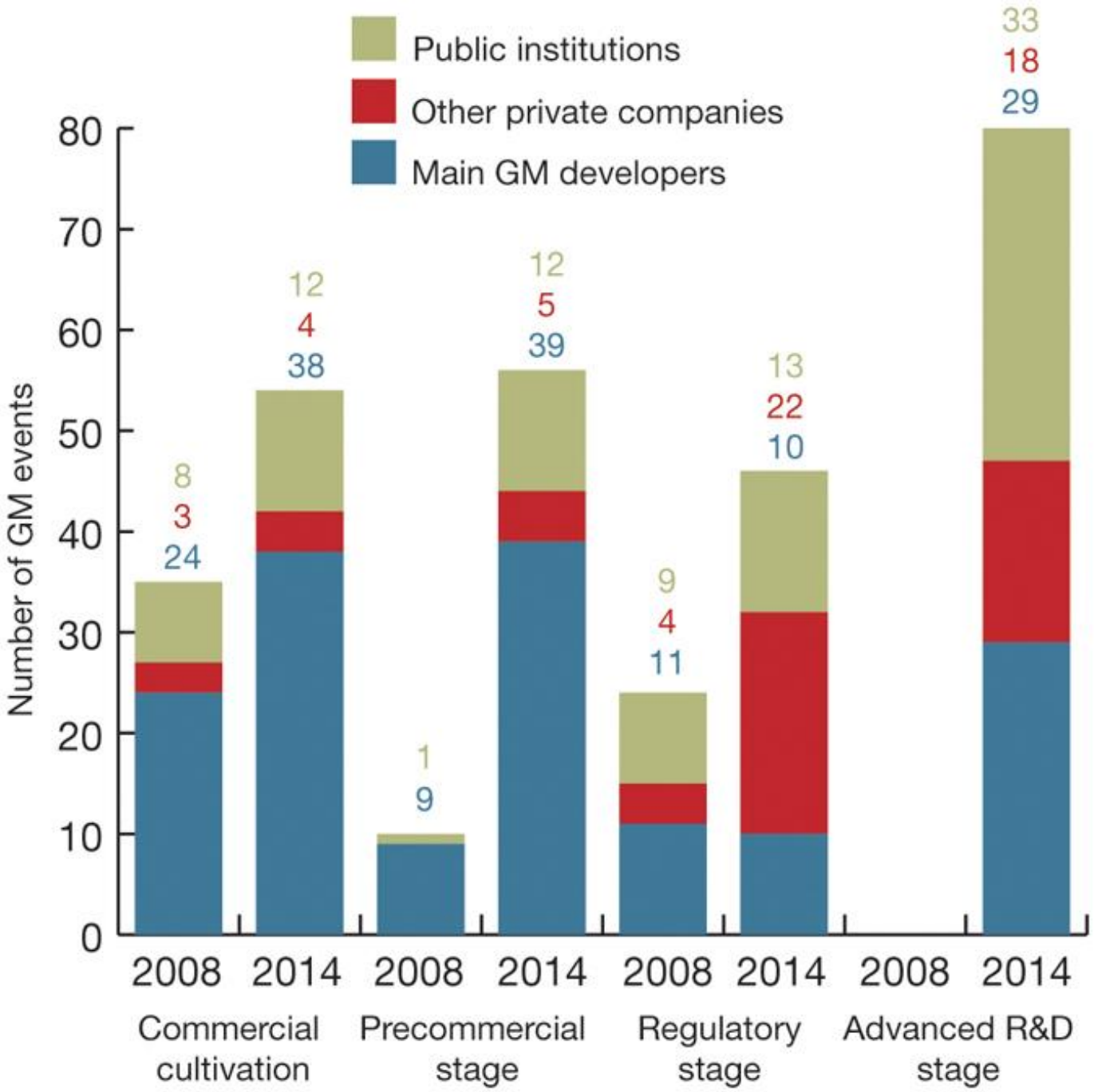


Figure 1

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.


Public institutions
Other private companies
Main GM developers
Number of GM events
0
10
20
30
40
50
60
70
80
8
3
24
12
4
38
1
9
12
5
39
9
4
11
13
22
10
33
18
29
2008
2014
2008
2014
2008
2014
2008
2014
Commercial cultivation
Precommercial stage
Regulatory stage
Advanced R&D stage

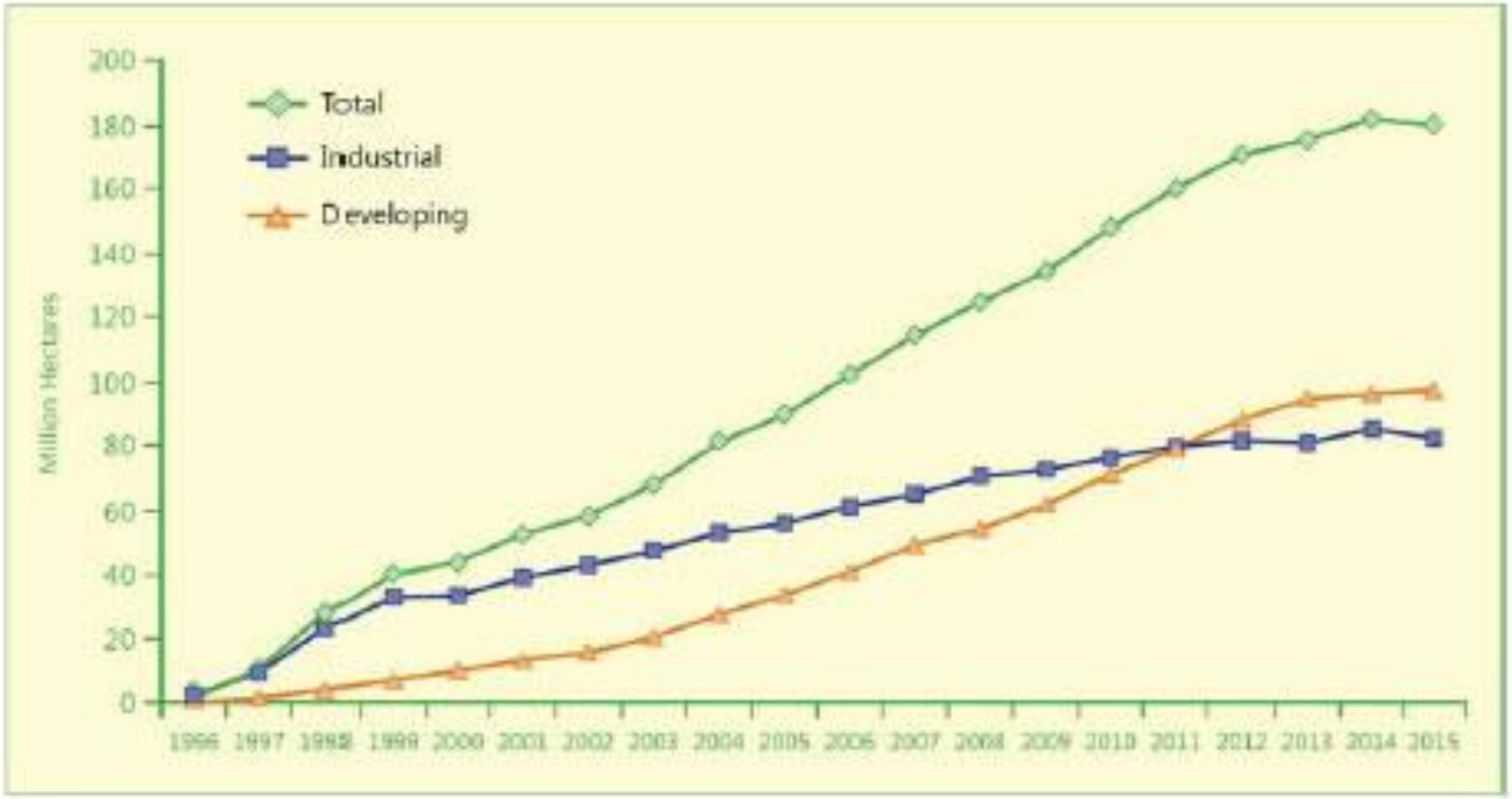


Figure 2. Global Area of Biotech Crops, 1996 to 2015: Industrial and Developing Countries (Million Hectares)

Source: Clive James, 2015.

Figure 1. Global Area of Biotech Crops, 1996 to 2015 (million hectares).

Source: Clive James, 2015.

















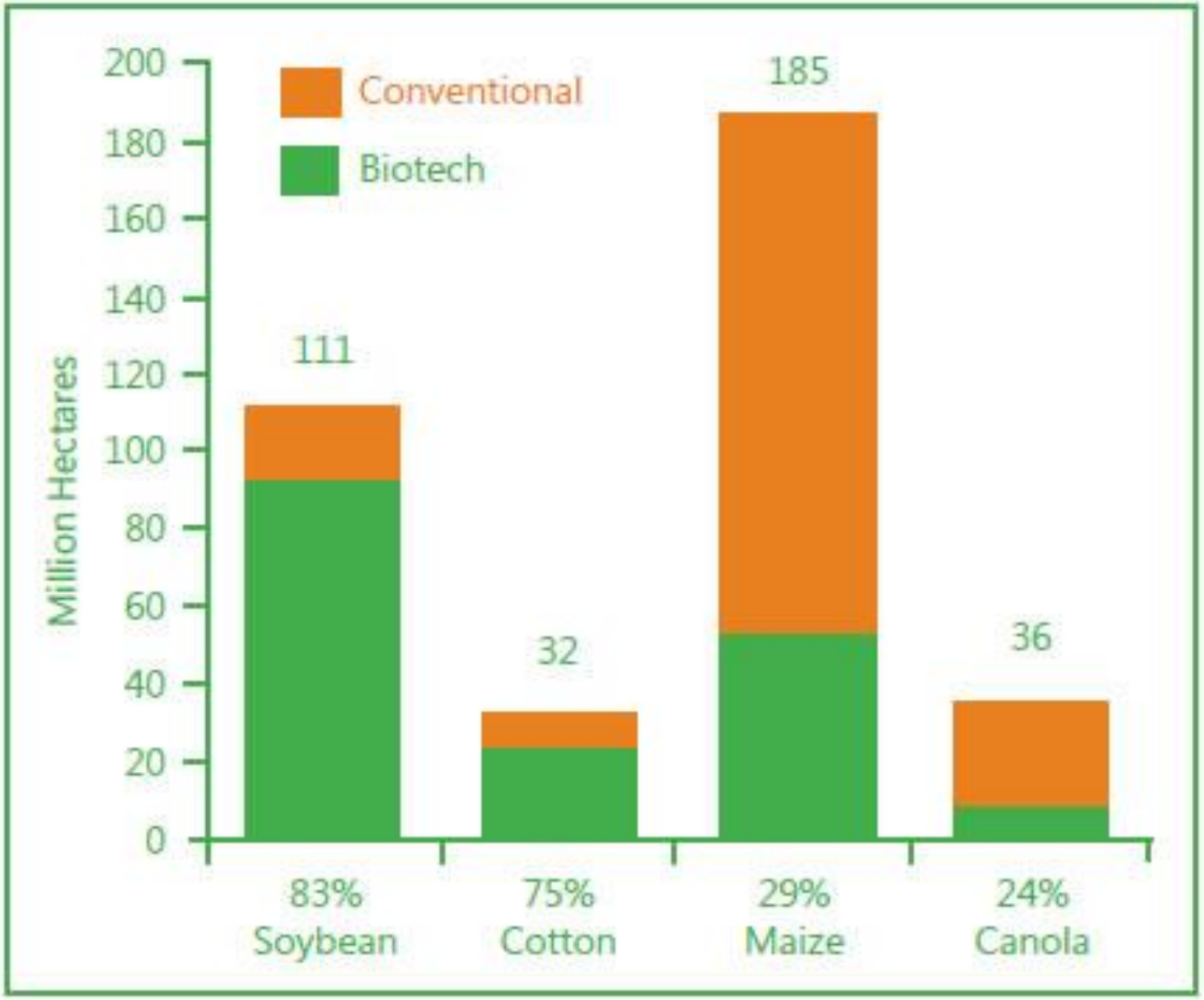


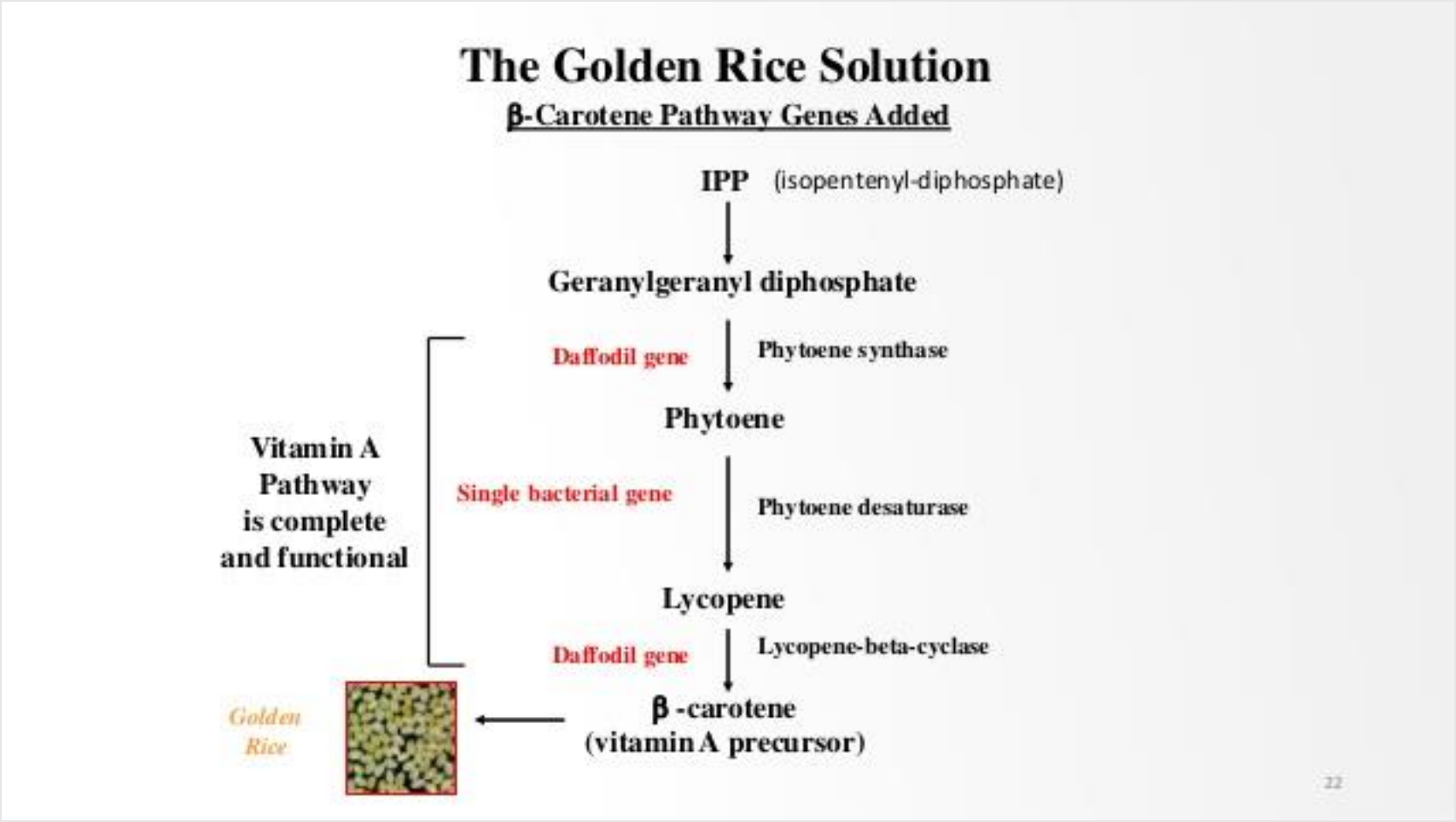
Figure 3. Biotech Crop Area as % of Global Area of Principal Crops, 2015 (Million Hectares)

Global Hectarages Data for 2015 (FAO, 2013)
Source: Compiled by Clive James, 2015.

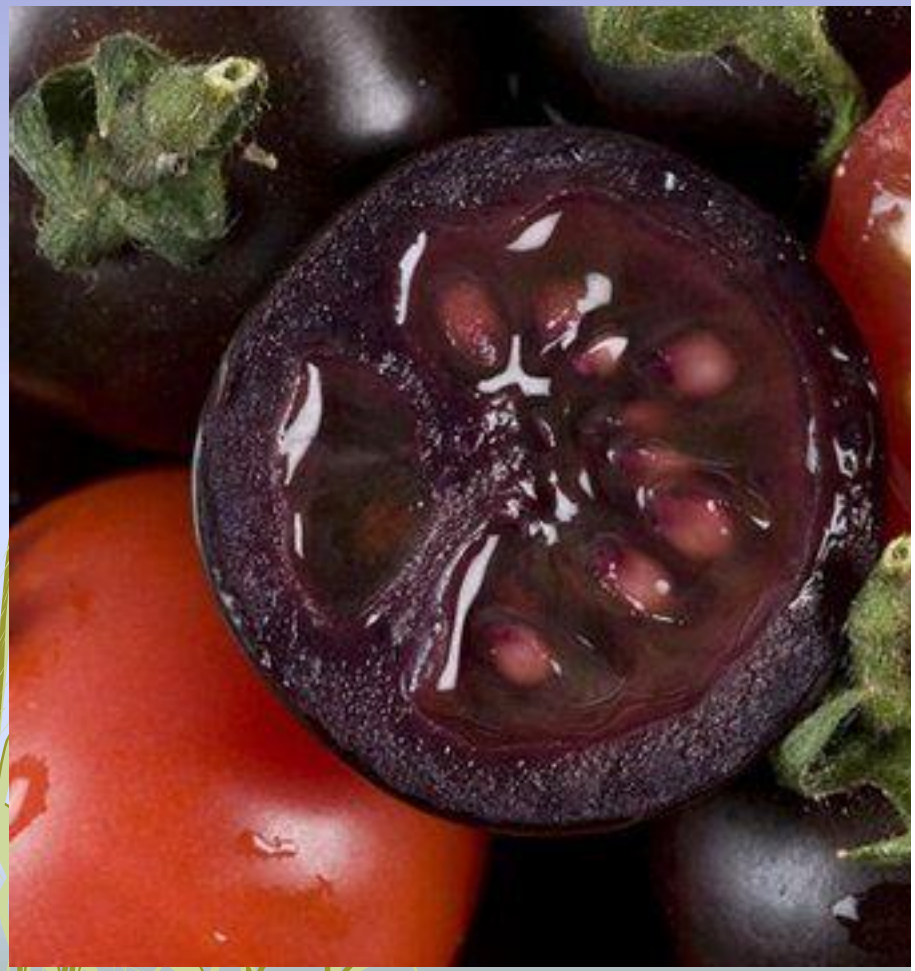
Table 1 Overview of the global pipeline of GM crops 2014[a]

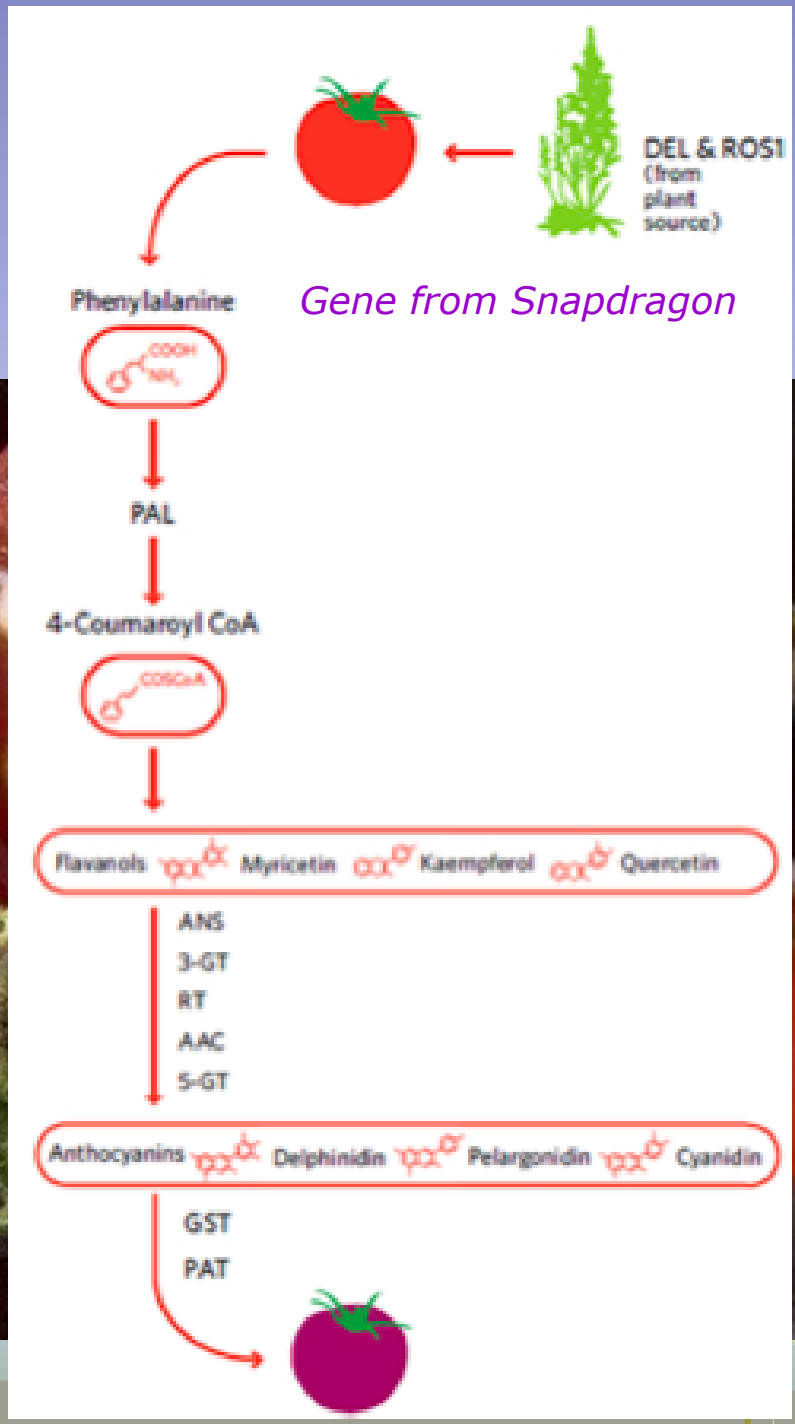
Crop	Stage					Type of traits		
	Commercial	Precommercial	Regulatory	Advanced development	Total events	Agronomic	Agronomic + quality	Quality
Cotton	16	6	3	5	30	29	0	0
Maize	15	8	1	6	30	28	0	2
Soybeans	5	10	4	12	31	27	2	2
OSR (oil seed rape)	3	9	0	8	20	16	0	4
Fruits (tree)	2	2	2	4	10	7	0	3
Vegetable	3	0	2	6	11	9	0	2
Alfalfa	2	0	1	0	3	2	0	1
Rice	1	4	1	17	23	18	0	5
Industrial crops	1	1	13	5	20	16	0	4
Sugar beet	1	1	0	1	3	3	0	0
Potato	0	10	11	2	23	12	0	11
Sugarcane	0	1	1	3	5	5	0	3
Leguminous crops	0	1	0	4	5	3	0	0
Cereals (others)	0	0	3	3	6	5	0	1
Fruits (ground)	0	0	1	1	2	3	1	0
Total	49	53	43	77	222	N.D.	N.D.	N.D.

Golden rice


The Golden Rice Solution
β-Carotene Pathway Genes Added
IPP (isopentenyl-diphosphate)
Geranylgeranyl diphosphate
Daffodil gene
Phytoene synthase
Phytoene
Vitamin A Pathway is complete and functional
Single bacterial gene
Phytoene desaturase
Lycopene
Daffodil gene
Lycopene-beta-cyclase
β -carotene (vitamin A precursor)
Golden Rice
22

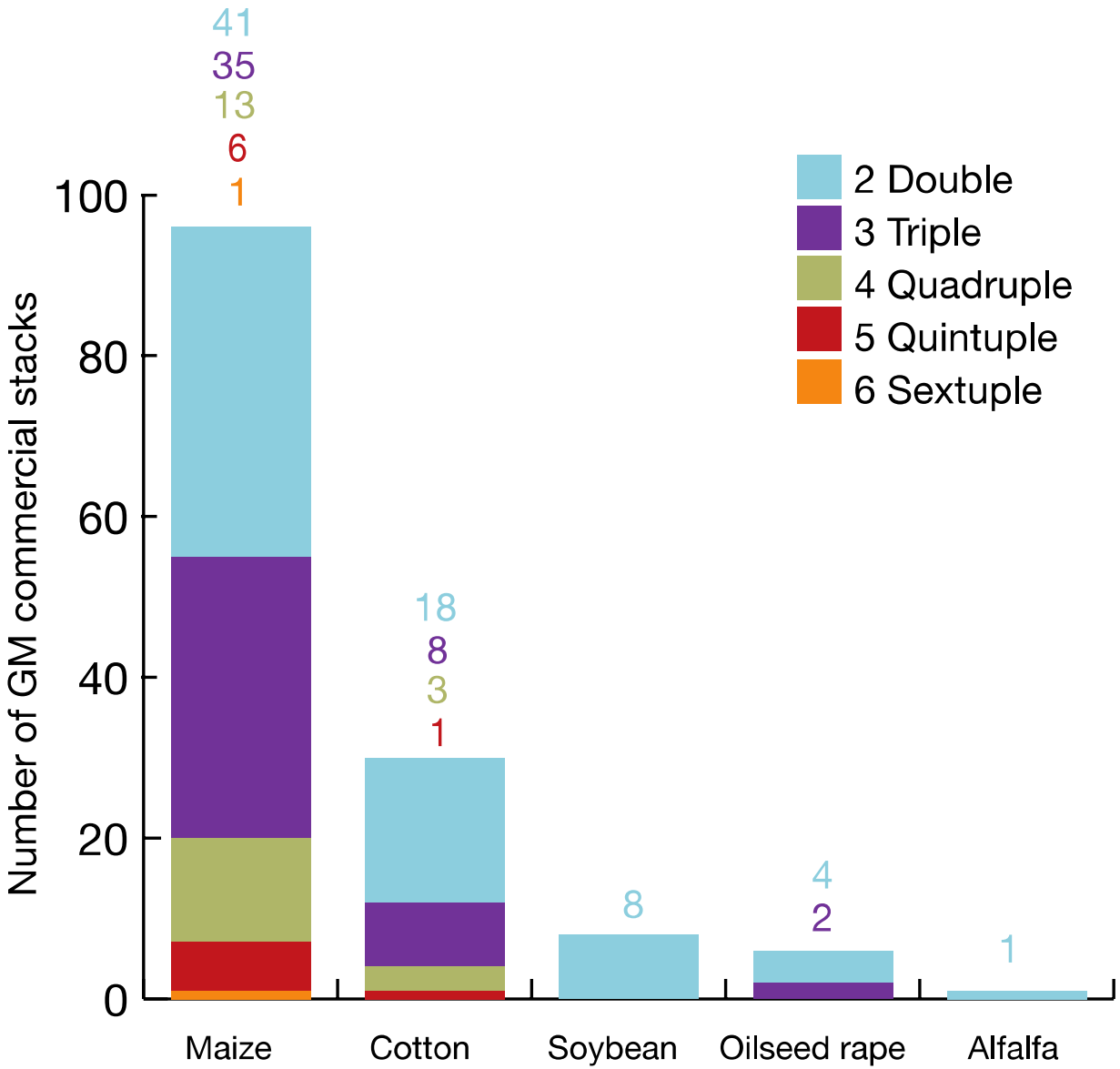
Purple tomato





Traits

- Improve shelf life
- Improve nutrition
- Stress resistance
- Herbicide resistance
- Pathogen resistance
- Production of biofuels
- Production of useful by products (drugs, antigens, materials, bioremediation)


41
35
13
6
1
18
8
3
1
8
4
2
1
2 Double
3 Triple
4 Quadruple
5 Quintuple
6 Sextuple
Number of GM commercial stacks
0
20
40
60
80
100
Maize
Cotton
Soybean
Oilseed rape
Alfalfa

BIOTECHNOLOGY

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered using CRISPR–Cas9 can be cultivated and sold without oversight.

BY EMILY WALTZ

STUART MCCALL/GETTY

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.

The US Department of Agriculture (USDA) will not regulate a mushroom that has been genetically modified with the gene-editing tool CRISPR–Cas9, the agency has confirmed. The long-awaited decision means that the mushroom can be cultivated and sold without passing through the agency's regulatory process — making it the first CRISPR-edited organism to receive a green light from the US government.

"The research community will be very happy with the news," says Caixia Gao, a plant biologist at the Chinese Academy of Sciences Institute of Genetics and Developmental Biology in Beijing, who was not involved in developing the mushroom. "I am confident we'll see more gene-edited crops falling outside of regulatory authority."

Yinong Yang, a plant pathologist at Pennsylvania State University (Penn State) in University Park, engineered the fungus — the common white button mushroom (*Agaricus bisporus*) — to resist browning. The effect is achieved by targeting the family of genes that encodes polyphenol oxidase (PPO), an enzyme that causes browning. By deleting just a handful of base pairs in the mushroom's genome, Yang knocked out one of six PPO genes — reducing the enzyme's activity by 30%.

AGENCY RULES

The mushroom is one of about 30 genetically modified organisms (GMOs) to sidestep the USDA's regulatory system in the past 5 years. In each case, the agency's Animal and Plant Health Inspection Service (APHIS) has said that the organisms — mostly plants — do not qualify as something that the agency must regulate. (Once a crop passes the USDA reviews, it may still undergo a voluntary review by the US Food and Drug Administration.)

Several of the plants that bypassed the USDA were made using gene-editing techniques such as the zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) systems. But until now, it was not clear whether the USDA would give the same pass to organisms engineered with science's hottest tool, CRISPR–Cas9.

Yang first presented the crop to a small group of USDA regulators in October 2015, after being encouraged to do so by an APHIS official. "They were very excited," Yang says: "There was certainly interest and a positive feeling" at the meetings. He followed up with an official letter of enquiry to the agency later that month.

The USDA's answer came this week. "APHIS does not consider CRISPR/Cas9-edited white button mushrooms as described in your October 30, 2015 letter to be regulated," the agency wrote to Yang on 13 April.

"I am confident we'll see more gene-edited crops falling outside of regulatory authority."

Yang's mushroom did not trigger USDA oversight because it does not contain foreign DNA from 'plant pests' such as viruses or bacteria. Such organisms were necessary for genetically modifying plants and fungi in the 1980s and 1990s, when the US government developed its framework for regulating GMOs. But newer gene-editing techniques that do not involve plant pests are quickly supplanting the old tools.

The United States is revamping its rules for regulating GMOs, which collectively are known as the Coordinated Framework for Regulation of Biotechnology. To that end, the US National Academies of Sciences, Engineering and Medicine have convened a committee that is charged with predicting what advances will be made in biotechnology products over the next five to ten years. It will hold its first meeting on 18 April.

In the meantime, Yang is mulling over whether to start a company to commercialize his modified mushroom. Fruits and vegetables that resist browning are valuable because they keep their colour longer when sliced, which lengthens their shelf life. In the past 18 months, biotech companies have commercialized genetically engineered non-browning apples and potatoes.

"I need to talk to my dean about that. We'll have to see what the university wants to do next," says Yang about the prospect of bringing his mushroom to market. But he notes that in September 2015, Penn State filed a provisional patent application on the technology. ■

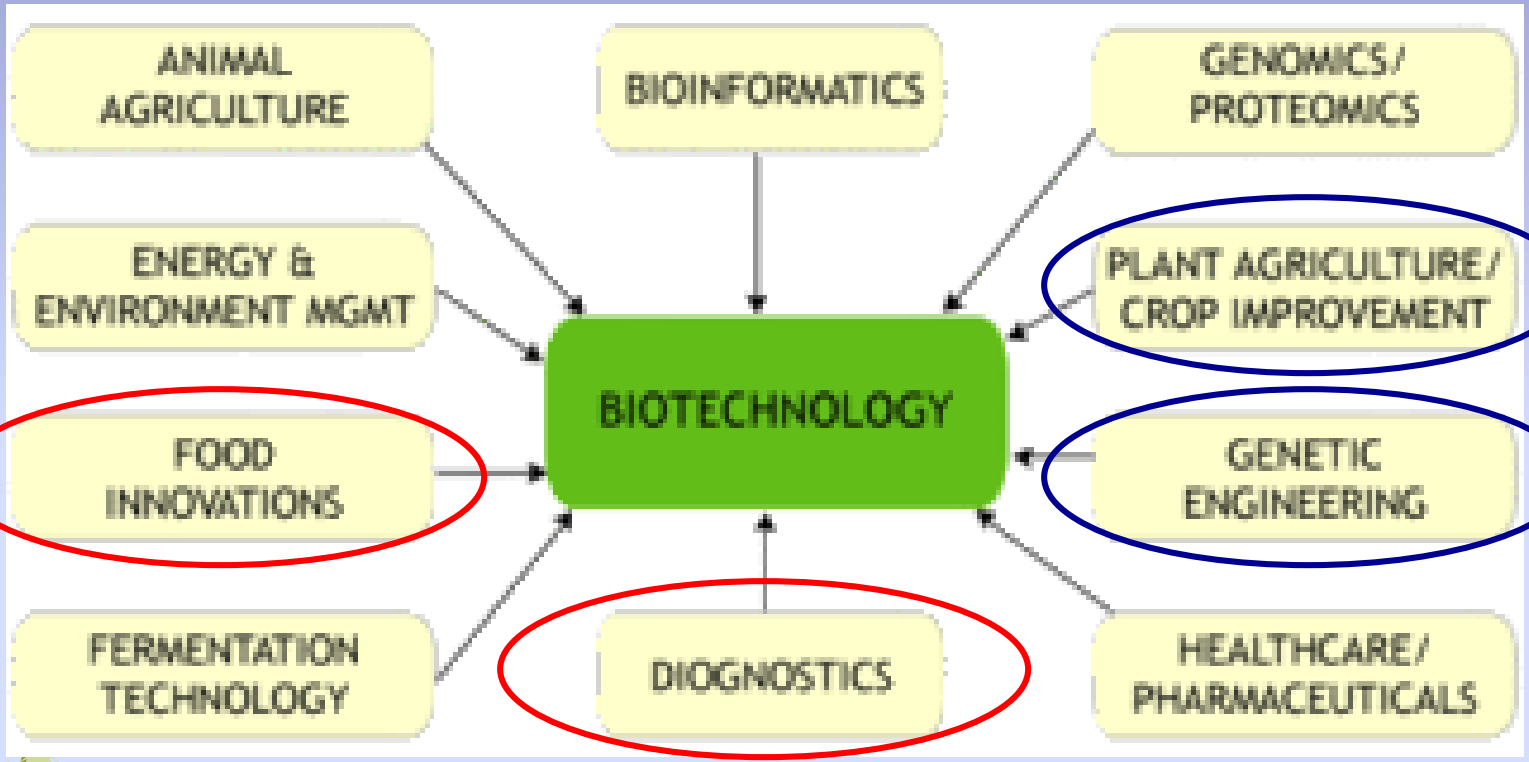
- Button mushroom
- CRISPR
- Polyphenol oxidase (PPO)
- Resist browning
- US regulation

The techniques under evaluation of US and European Commission :

- *Site-Directed Nucleases (SDN) including Zinc finger nuclease technology, CRISPR and TALENs*
- *Oligonucleotide-directed mutagenesis*
- RNA interference (RNAi)*
- *Cisgenesis*
- *Intragenesis*
- *Grafting*
- *Agro-infiltration*
- *RNA-dependent DNA methylation*
- *Reverse breeding*

* Only for USA

http://gmoinquiry.timelinesproject.org/FAQ/how-are-governments-regulating-crispr-and-new-breeding-technologies-nbts/
http://beyond-gm.org/gmo-or-gm-no-how-will-the-eu-regulate-new-plant-breeding-technologies/


ANIMAL AGRICULTURE
BIOINFORMATICS
GENOMICS/ PROTEOMICS
ENERGY & ENVIRONMENT MGMT
PLANT AGRICULTURE/ CROP IMPROVEMENT
BIOTECHNOLOGY
FOOD INNOVATIONS
GENETIC ENGINEERING
FERMENTATION TECHNOLOGY
DIOGNOSTICS
HEALTHCARE/ PHARMACEUTICALS

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

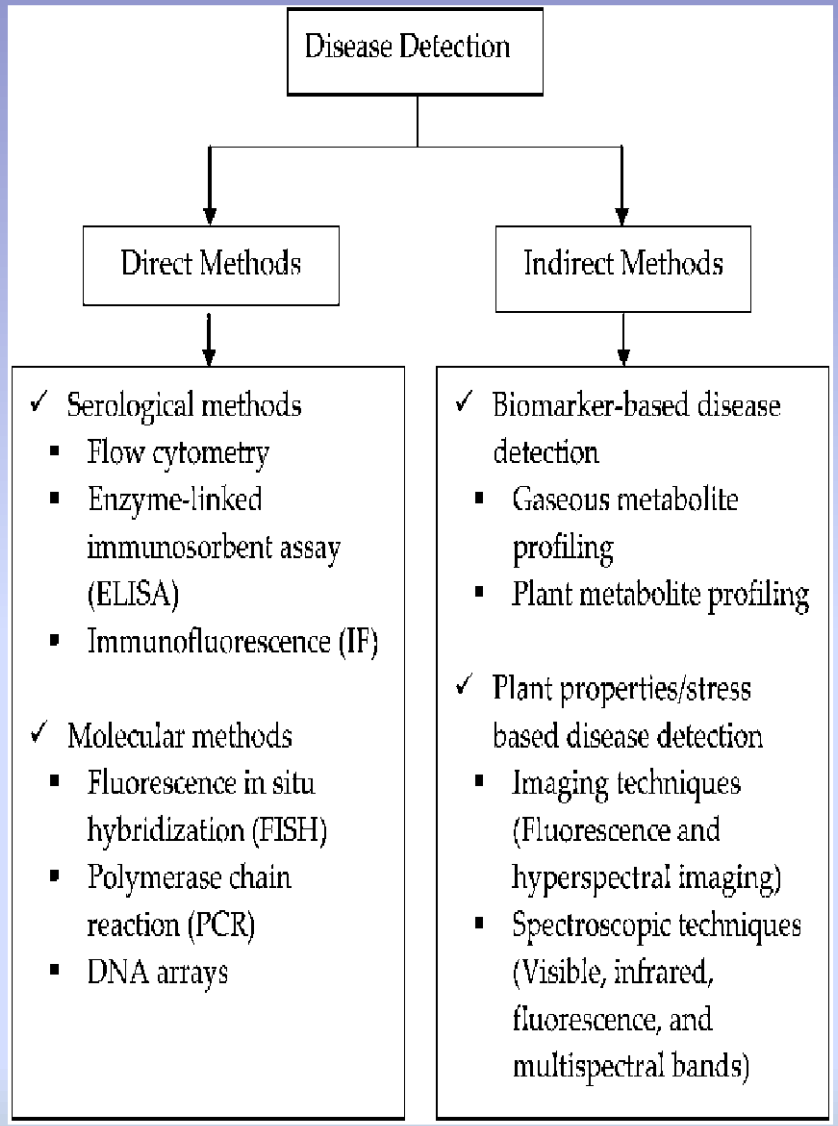


Fig. 1. Methods of plant disease detection.

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)
Onion	*Sclerotium cepivorum*	Fungi	PCR	Anwar Haq et al. (2003)
Olive	*Pseudomonas savastanoi* pv. *savastanoi*.	Bacteria	PCR, Hybridization	Bertolini et al. (2003)
Sweet orange	*Candidatus* Liberibacter asiaticus.	Bacteria	PCR	Das (2004)
Sweet orange	*Candidatus Liberibacter asiaticus*, *Ca. L. americanus*, *Ca. L. africanus*	Bacteria	PCR	Teixeira et al. (2005)
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)

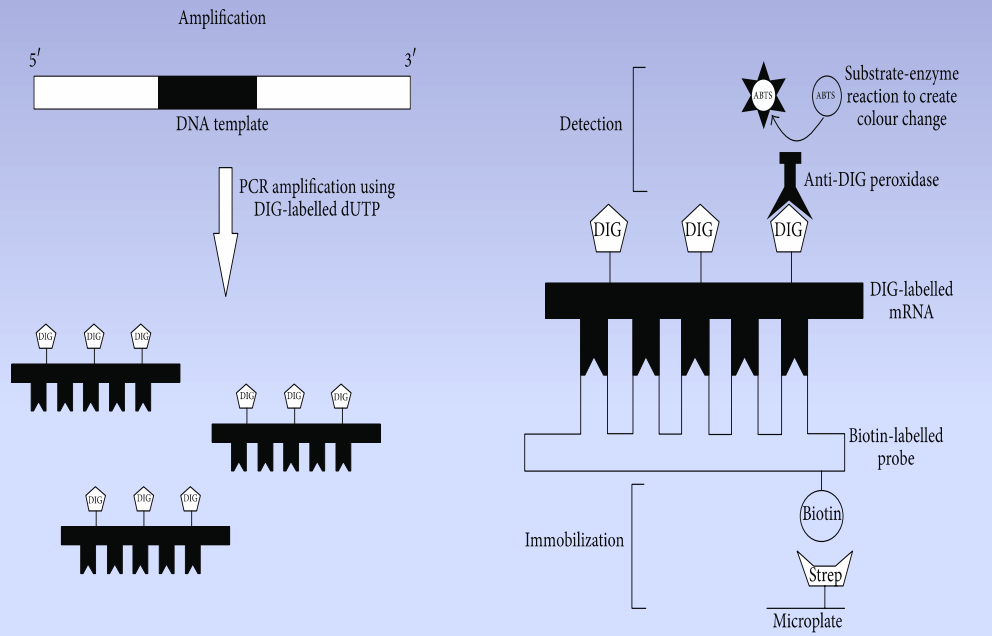


Figure 1: Illustration of the 3-step PCR-ELISA method: (i) amplification of the gene of interest using PCR in the presence of DIG-dUTP, which is then bound to specific probes, (ii) immobilization of the gene of interest to the microplate through strong affinity of avidin-biotin interaction, followed by (iii) detection of biotinylated DNA using an anti-DIG-peroxidase conjugate with substrate ABTS to form a blue-green color reaction that is both visible and measured using a spectrophotometer.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 653014, 6 pages
http://dx.doi.org/10.1155/2014/653014

Table 1: Comparisons between 3 different detection methods; conventional PCR with agarose gel electrophoresis, PCR-ELISA and qPCR.

Comparison	Conventional PCR	PCR-ELISA	qPCR
Equipment required	Standard laboratory equipment	Standard laboratory equipment	Requires fluorescence detection instrument
Reagent costs	Low	Moderate	Costly
Detection limit	1–10 ng/μL	0.01 ng/μL	0.25 pg/μL
Quantitative ability	Not quantitative	Semi-quantitative	Quantitative

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BioMed Research International
Volume 2014, Article ID 653014, 6 pages
http://dx.doi.org/10.1155/2014/653014

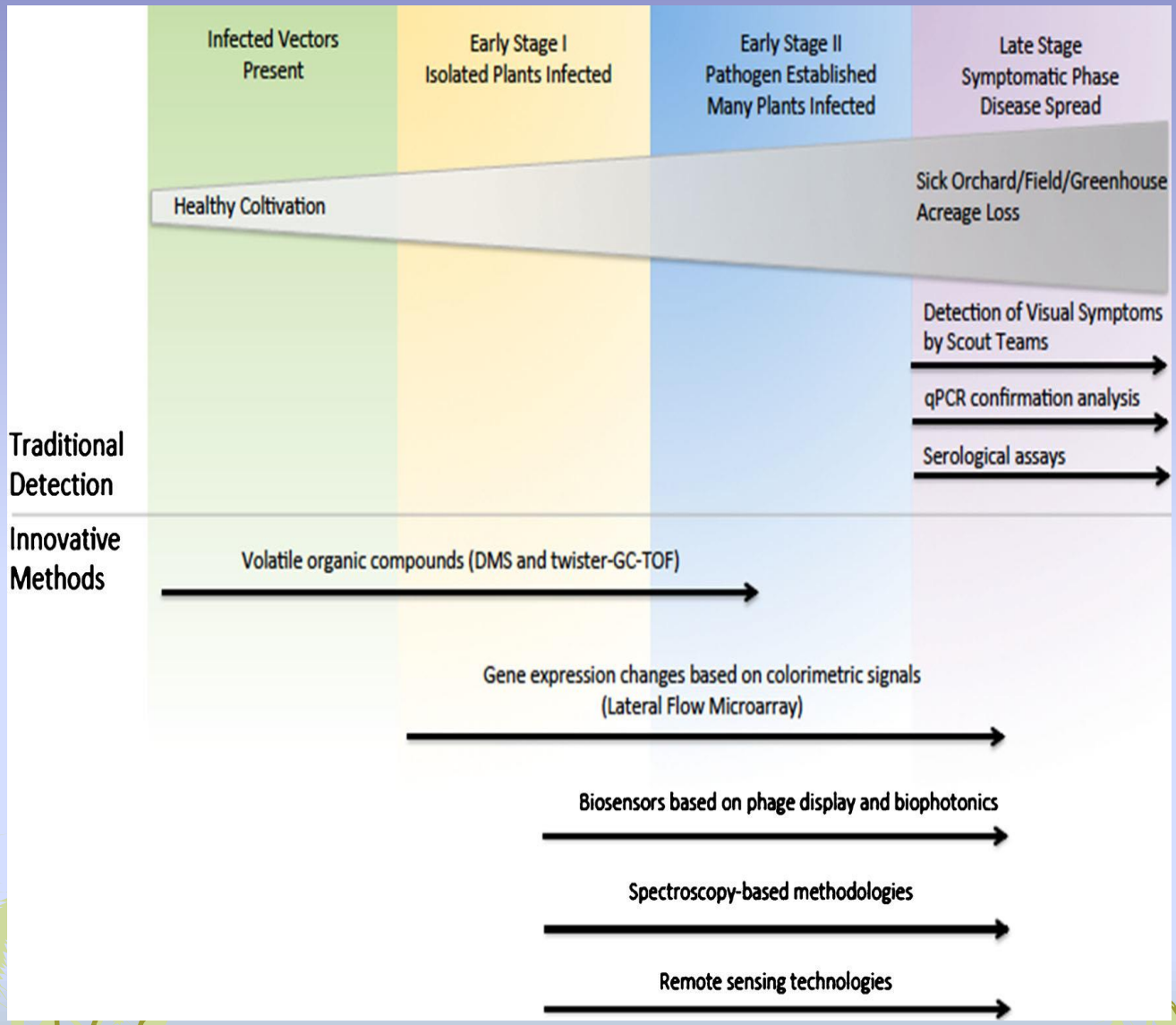
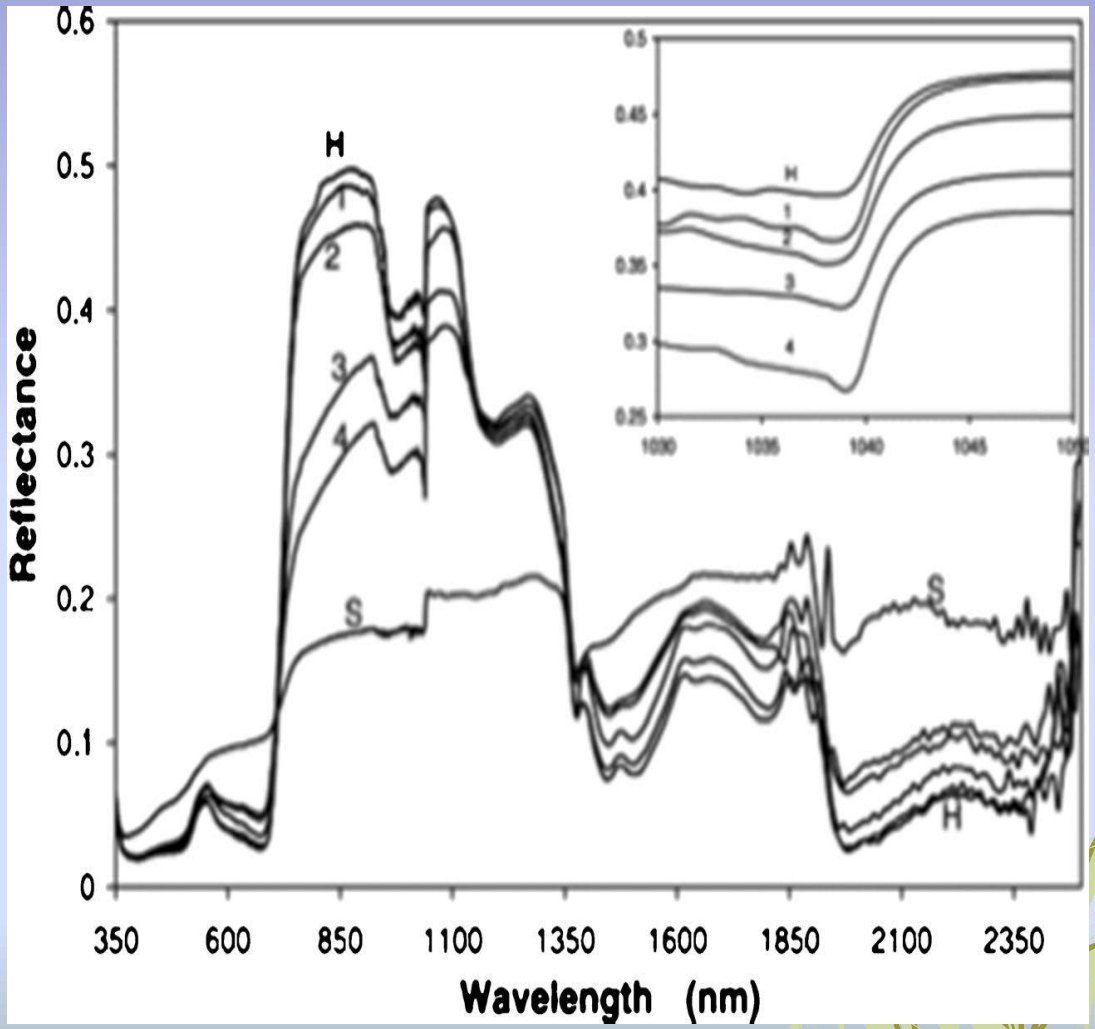


Fig. 3 Traditional and innovative methods. Their timing of use during plant disease progression was indicated. Four disease stages were considered

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)



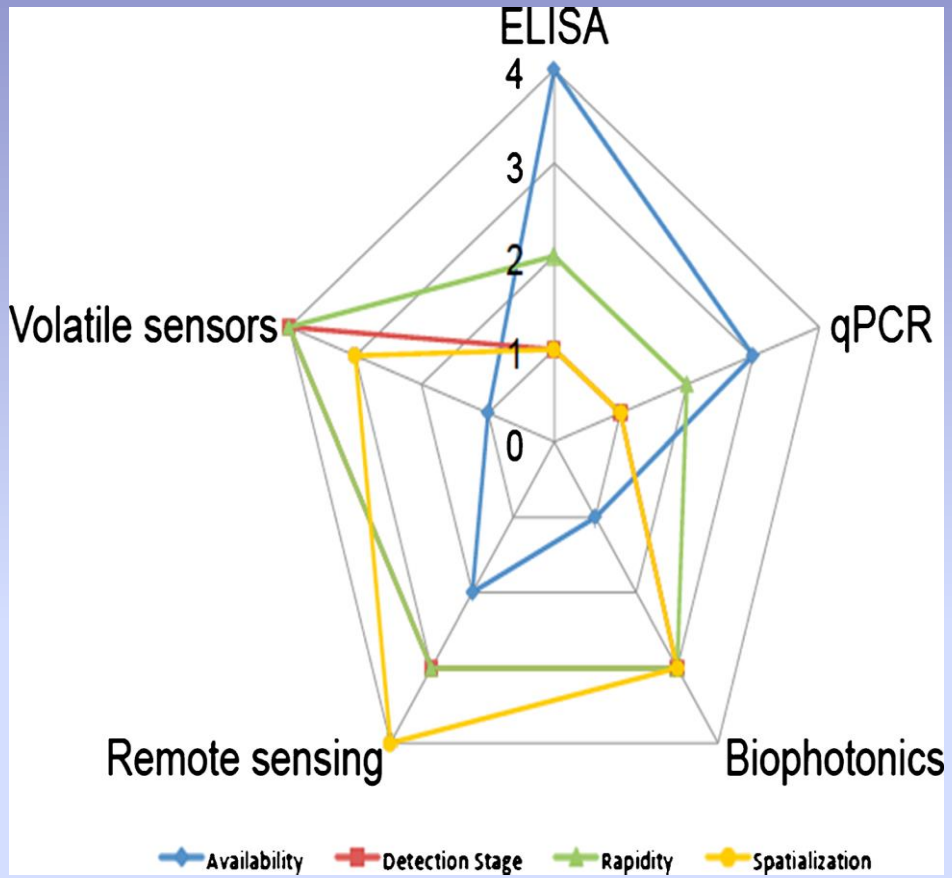


Fig. 7 Comparison of methods for plant disease detection (PDD). The qualitative scales indicate: 1 poor, 2 fair, 3 good, and 4 very good. The categories evaluate individual techniques with respect to: (i) Availability—ease of use, availability of equipment, and cost; (ii) detection stage—when infections 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

spices and condiment

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

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)

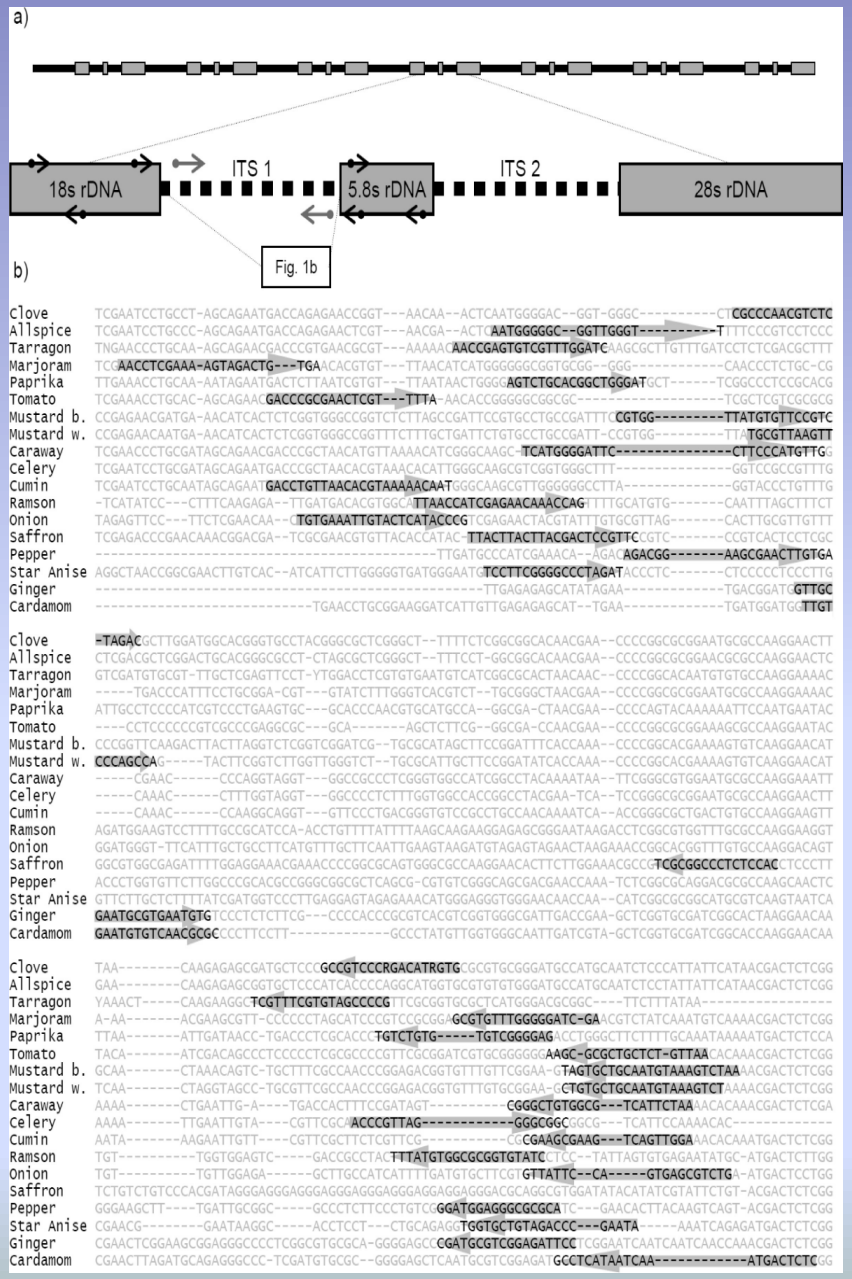


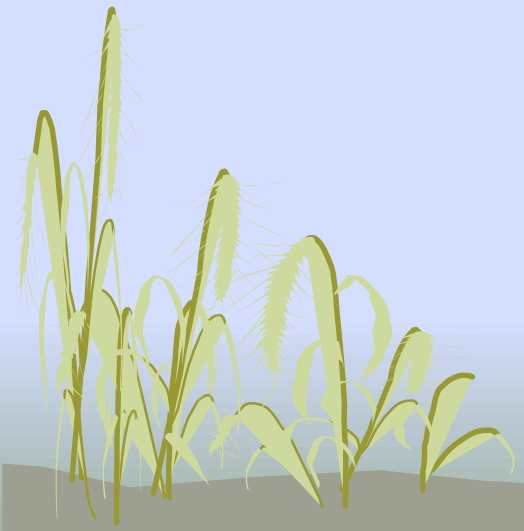
Figure 1. rDNA sequences and primer binding sites. (**a**) Gray boxes: rDNA genes (18s, 5.8s, and 28s), dotted lines ITS 1 and 2; arrows: primer binding sites (gray, specific; black, universal). (**b**) Sequence alignment of ITS1 created with ClustalX v. 2.0.9 (*20*) with primer binding sites. Gray arrows 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.

FOOD INNOVATION




Green Giant
Sweet Peas
KEEP FROZEN
Sweet Peas

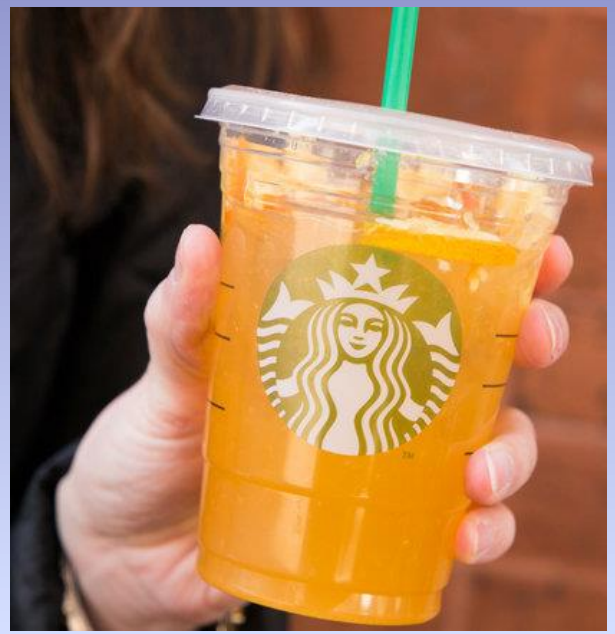
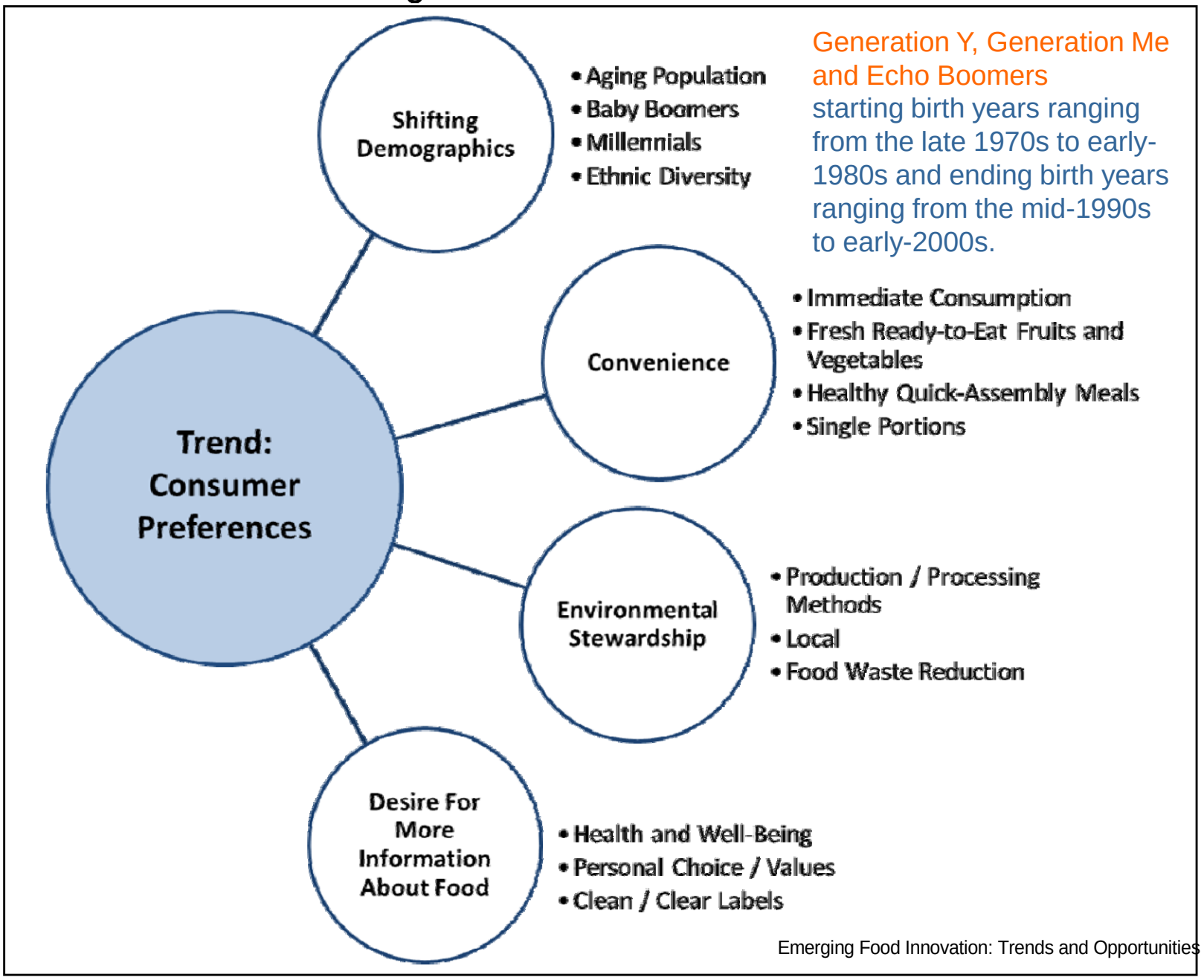


Figure 2. Consumer Preferences



Emerging Food Innovation: Trends and Opportunities

Figure 3. Marketplace Pressures

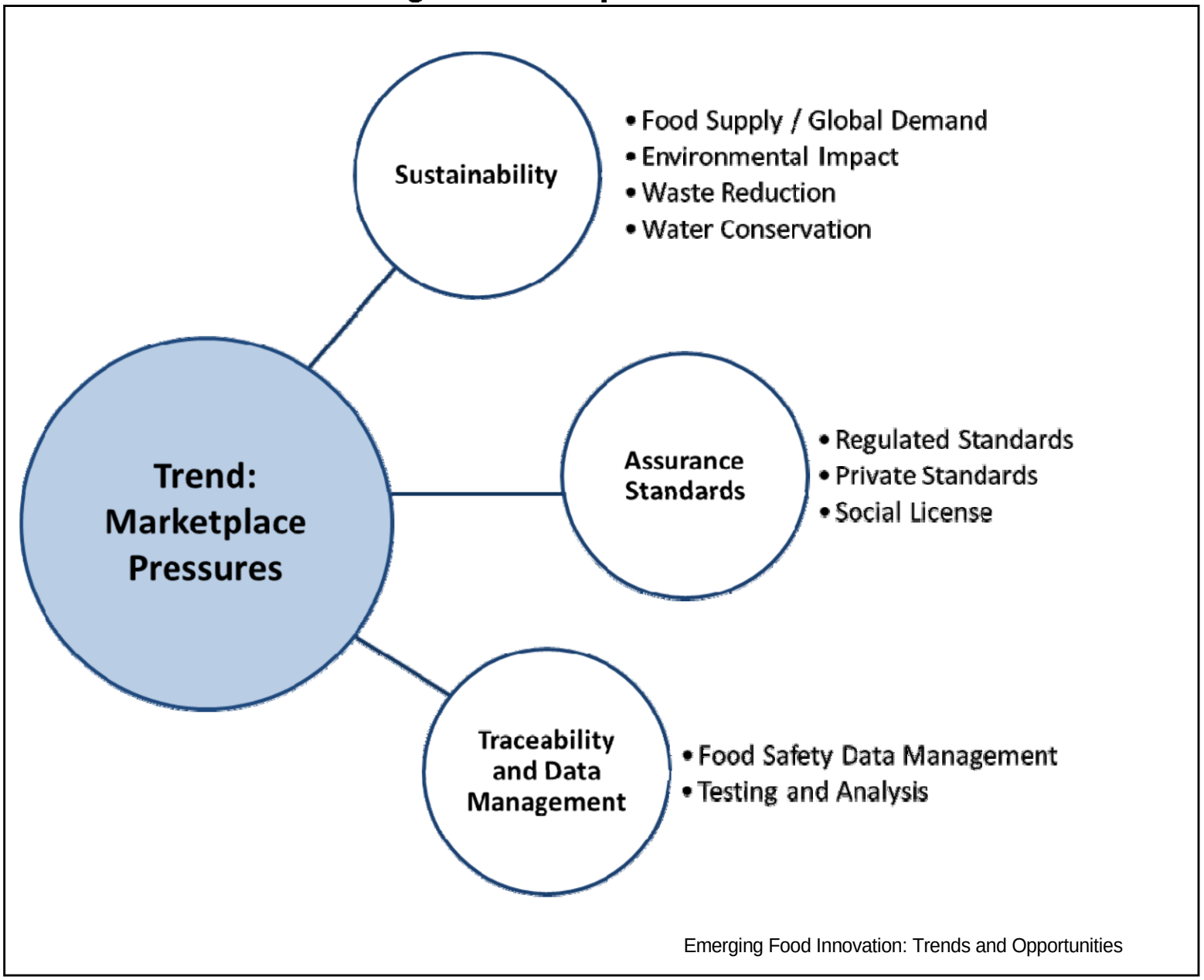


Figure 4. Innovative Ingredients

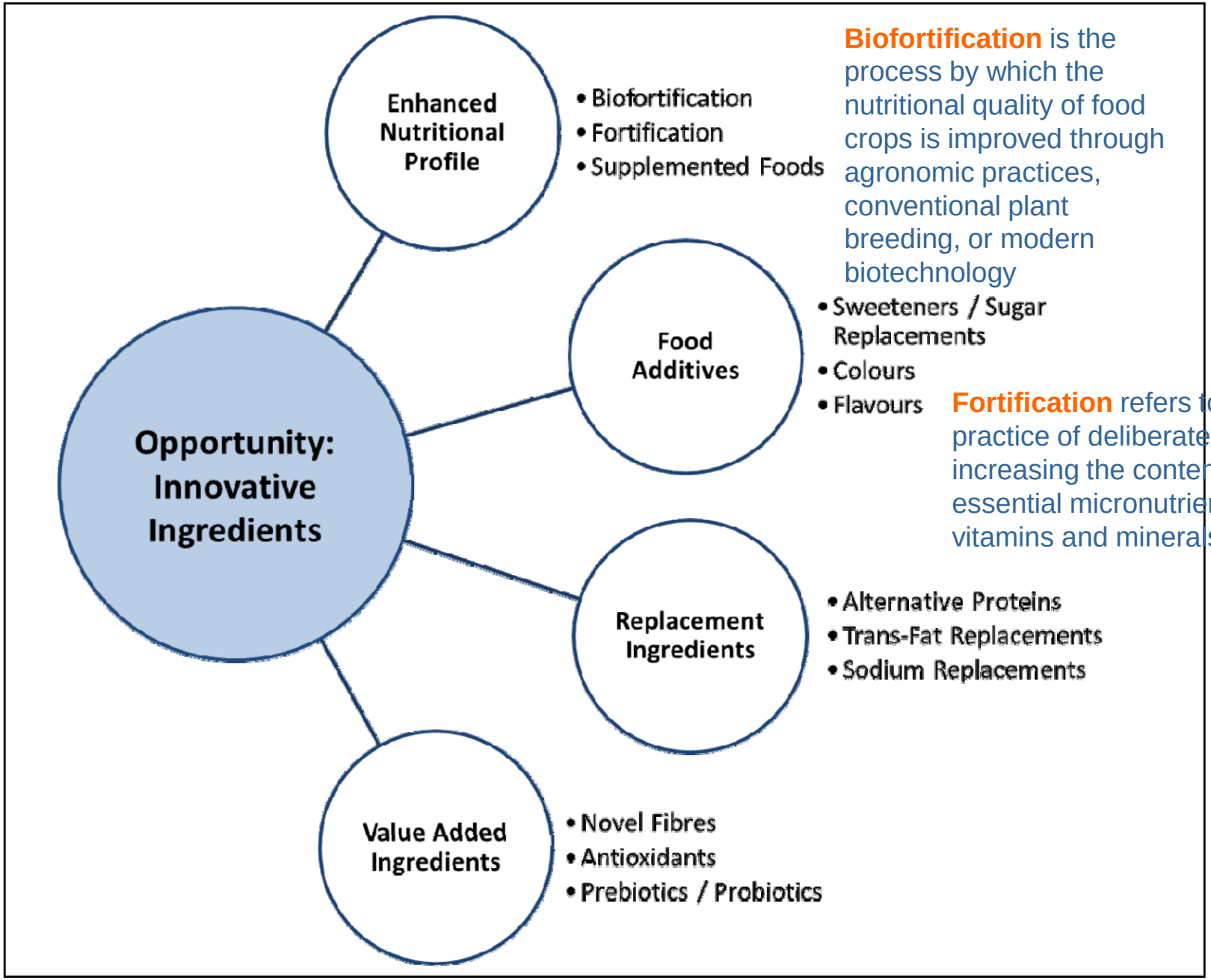


Figure 5. Emerging Technologies

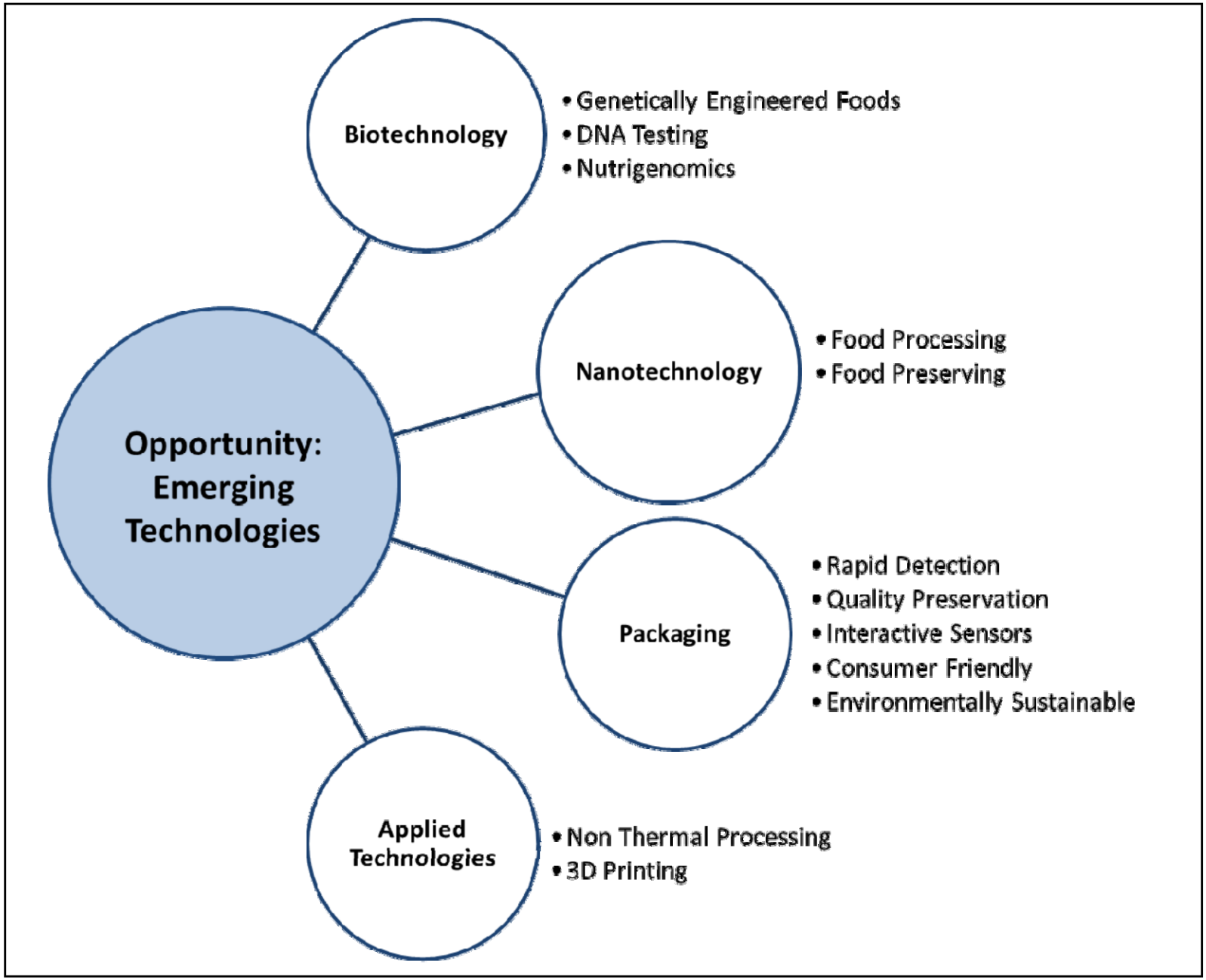
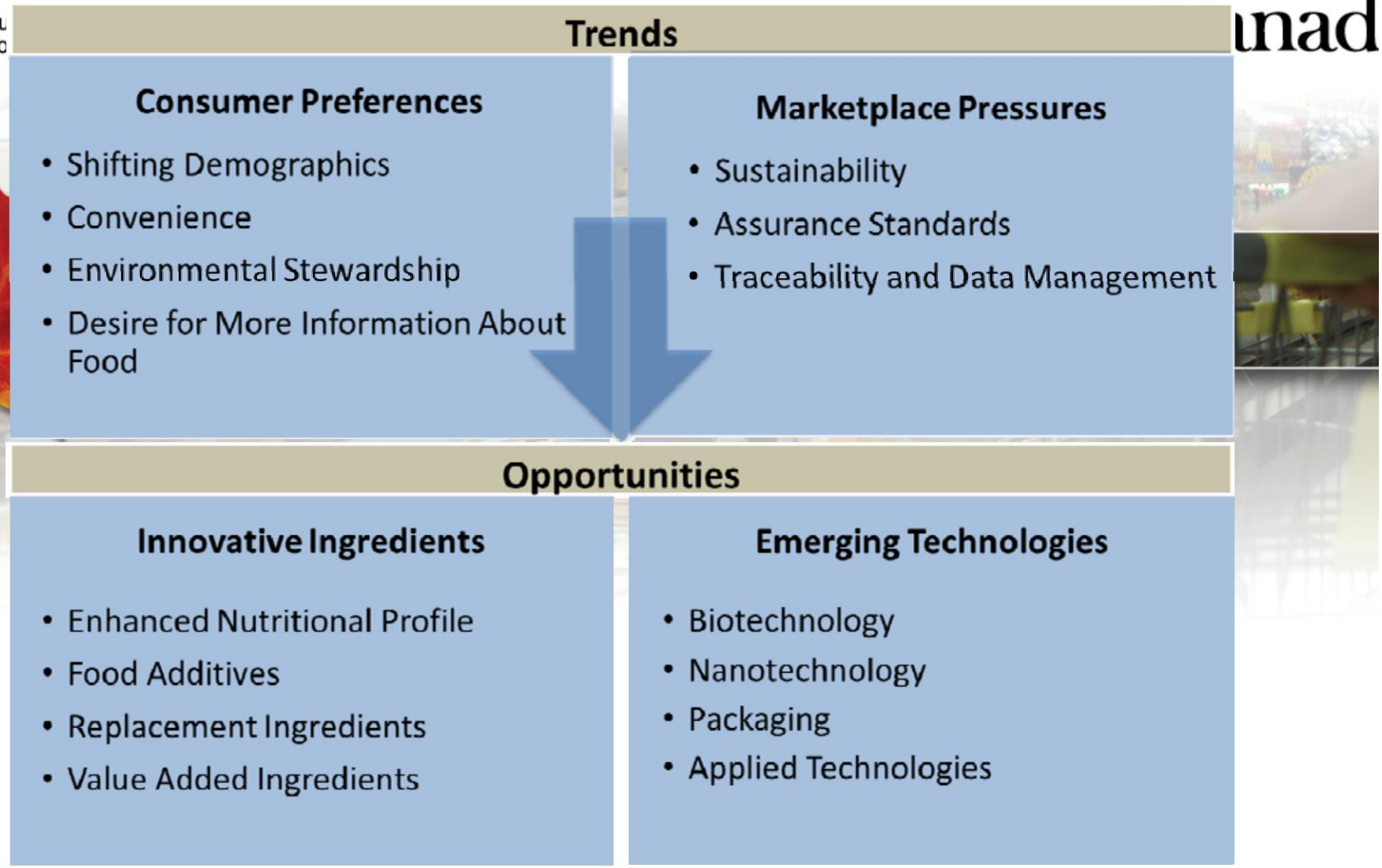
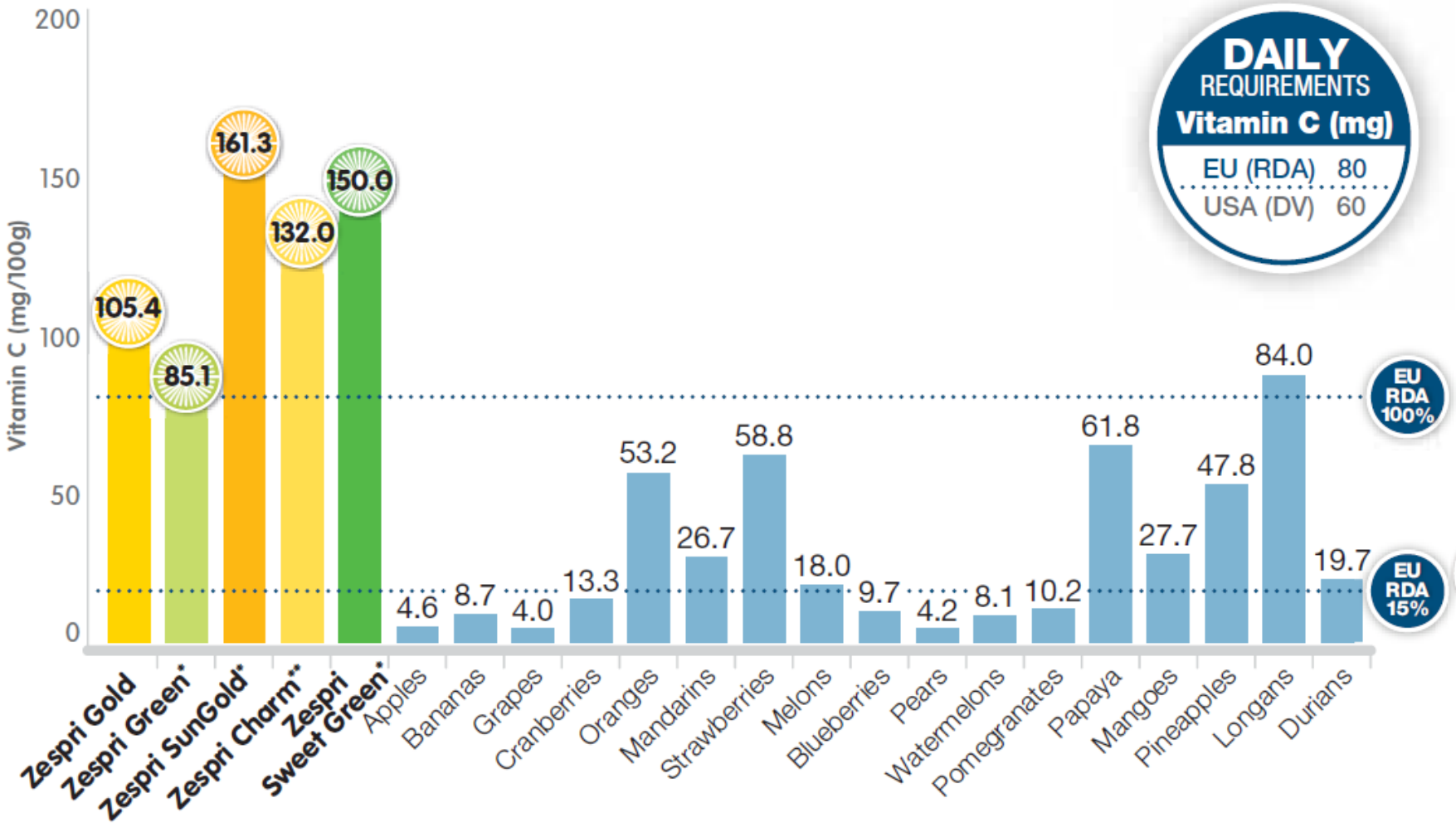


Figure 1. Trends and Opportunities for the Food Processing Industry



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


Zespri
Green
Kiwifruit
Nutritious
Vitality


Zespri
Sweet Green
Kiwifruit
Deliciously
Sweet


Zespri
Charm
Kiwifruit
Tropically
Sweet


Zespri
SunGold
Kiwifruit
Refreshingly
Juicy


Zespri
Gold
Kiwifruit
Delicious
Vitality

3

CONCLUSION

What do we want from biotechnology to reduce postharvest losses

- **Premium line**
  - **tissue culture, molecular marker, genetic engineering**
- **Maintenance of premium lines**
  - **tissue culture, molecular markers**
- **Detection of impurity**
  - **diagnosis**

More…

- The means to have premium lines with resist to abiotic and biotic stress
- Use less resources (water, fertilizer etc)
- Produce less waste
- Future (and better) food


THANK YOU FOR YOUR ATTENTION