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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND DNA-PROFILING IN PARTICULAR

Thirteenth Session
Brasilia, November 22 to 24, 2011

ADDENDUM

DEVELOPMENT OF FUNCTIONAL MARKERS ASSOCIATED WITH PHENOTYPIC TRAITS FOR IDENTIFICATION OF RICE VARIETIES

Document prepared by experts from Uruguay

Development of functional markers associated with phenotypic traits for varietal identification in soybean and rice

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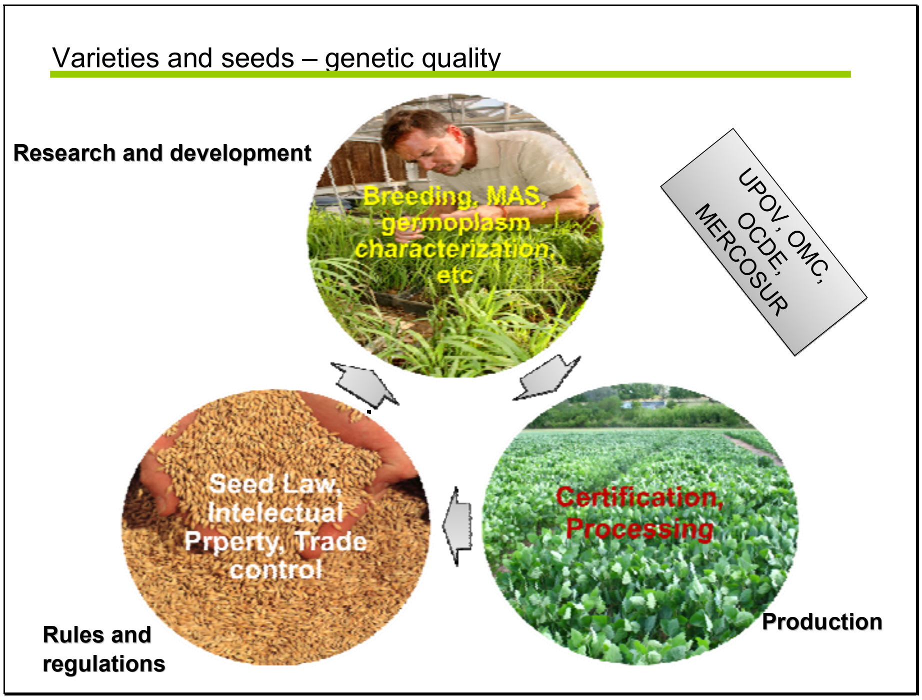
URUGUAY



Varieties and seeds – genetic quality

Research and development

Breeding, MAS, germoplasm characterization, etc

UPOV, OMC, OCDE, MERCOSUR

Certification, Processing

Production

Seed Law, Intelectual Prperty, Trade control

Rules and regulations

Characterization and discrimination of varieties

Description of varieties

DUS Test (UPOV-INASE guidelines)

Phenologics characteristics (height, fowering time, flower color, etc.)

Field trials and greenhouse

Laboratory

Morphological markers

Relatively easy to assess
They can be affected by the environment
Two or three growth cycles (time)
Use more stable characters (number limited)
Late manifestation of some characters
Infrastructure
Qualified human resources

Molecular markers – DNA profiles

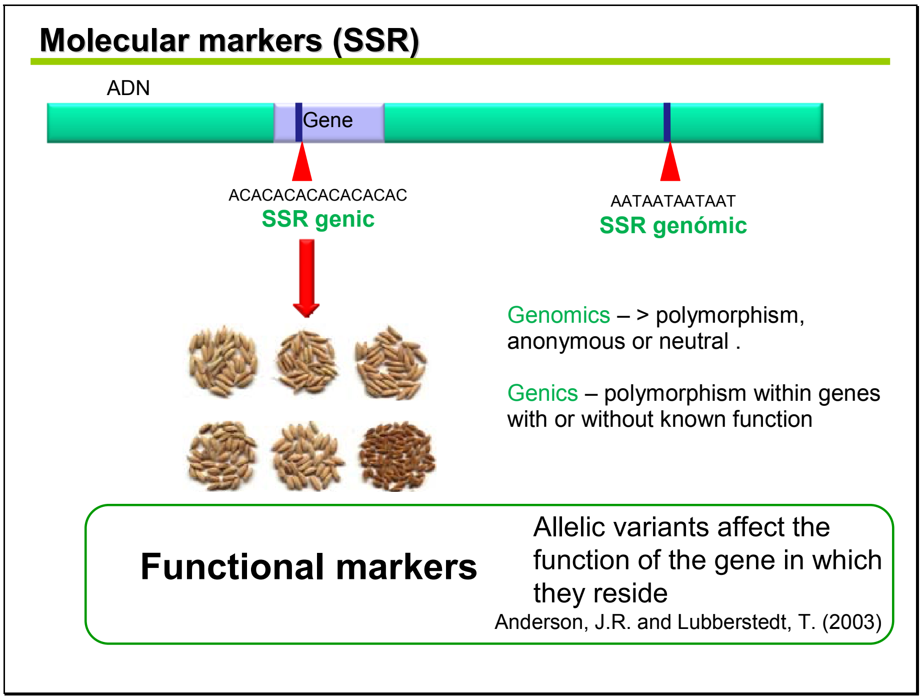
(fingerprinting)

Advantages

- Almost unlimited number
- Low environment effect
- Highly polymorphic
- Speed
- Different matrix (seeds, seedlings)
- Robust and reproducible
- Cost and accessible technology

SSRs, SNPs, RAPDs, AFLPs

- Lack of information on phenotypic expression of markers "anonymous“
- Difficult to use as a molecular descriptor for registration purposes for a new variety,
- The new variety have to be different from the others in at least one phenotypic characteristic.



Molecular markers (SSR)

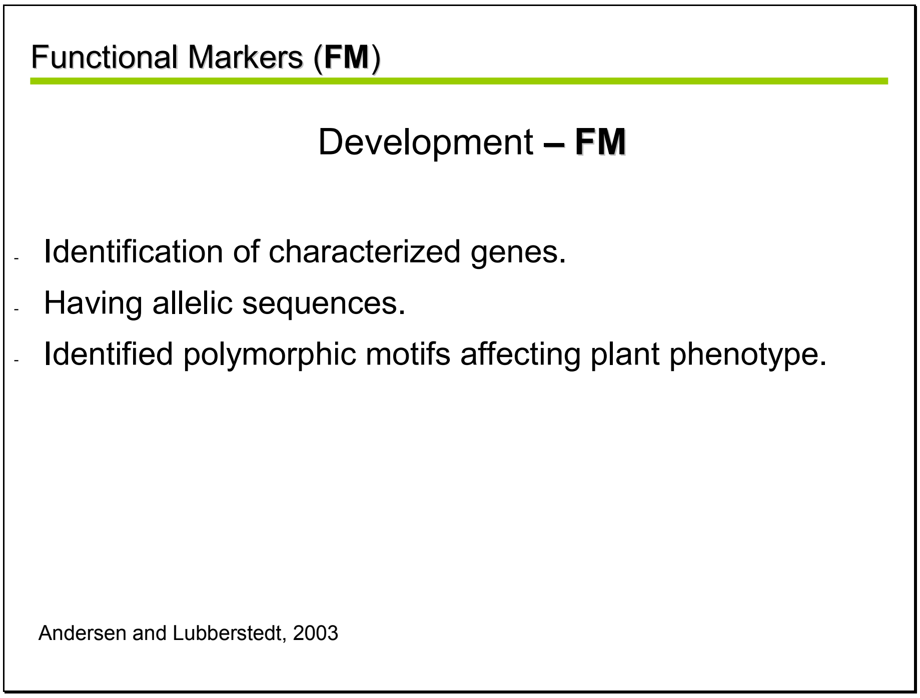
Genomics – > polymorphism, anonymous or neutral .

Genics – polymorphism within genes with or without known function

Functional markers

Allelic variants affect the function of the gene in which they reside

Anderson, J.R. and Lubberstedt, T. (2003)



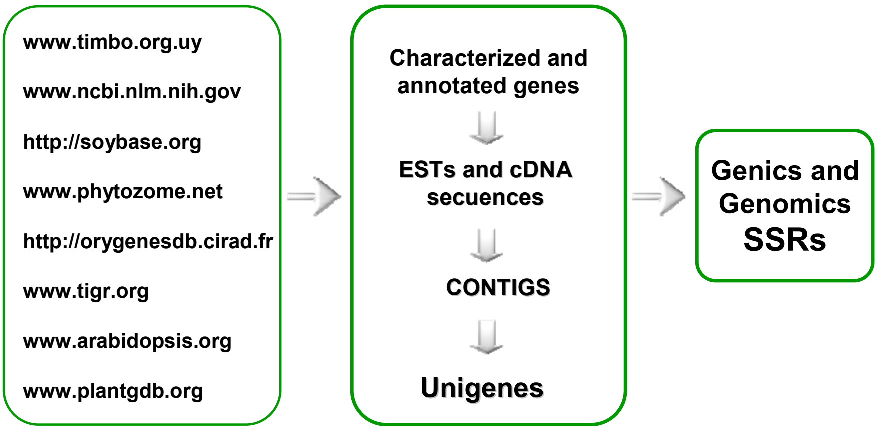
Functional Markers (**FM**)

Development – **FM**

- Identification of characterized genes.
- Having allelic sequences.
- Identified polymorphic motifs affecting plant phenotype.

Andersen and Lubberstedt, 2003

Resourses of secuences (bioinformatics tools)



www.timbo.org.uy
www.ncbi.nlm.nih.gov
http://soybase.org
www.phytozome.net
http://orygenesdb.cirad.fr
www.tigr.org
www.arabidopsis.org
www.plantgdb.org

Characterized and annotated genes
ESTs and cDNA secuences
CONTIGS
Unigenes

Genics and Genomics SSRs

Bioinformatic tools - Ontology

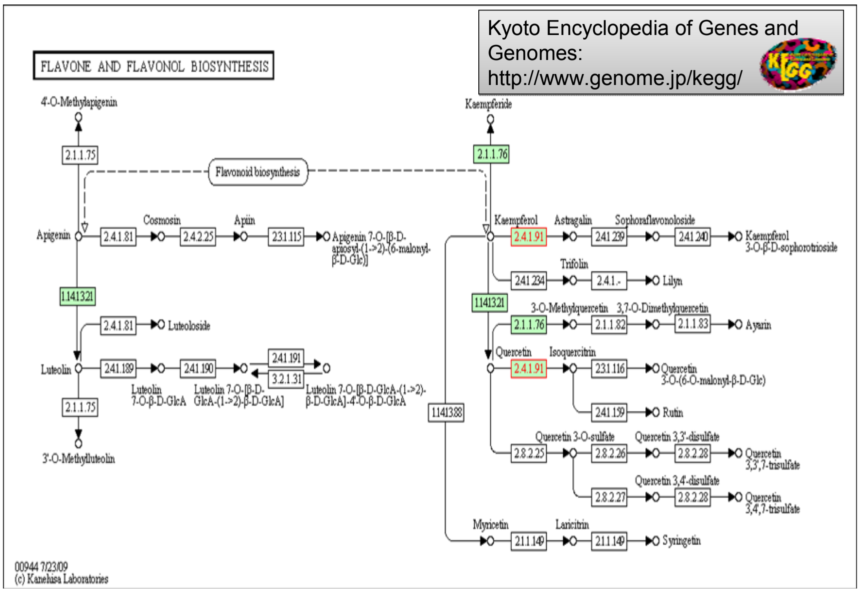
AmiGO

Classification of conceptual categories of gene (annotation terms of gene products)

http://www.geneontology.org

- ⊞ all : all [249595 gene products]
  - ⊞ I GO:0008150 : biological_process [175820 gene products]
    - ⊞ I GO:0032502 : developmental process [20961 gene products]
      - ⊞ I GO:0048532 : anatomical structure arrangement [201 gene products]
        - ⊞ I **GO:0010358 : leaf shaping** [5 gene products]
      - ⊞ I GO:0048856 : anatomical structure development [14349 gene products]
        - ⊞ P GO:0009653 : anatomical structure morphogenesis [7364 gene products]
          - ⊞ P GO:0048532 : anatomical structure arrangement [201 gene products]
            - ⊞ I **GO:0010358 : leaf shaping** [5 gene products]
          - ⊞ I GO:0009887 : organ morphogenesis [3816 gene products]
            - ⊞ I GO:0009965 : leaf morphogenesis [128 gene products]
              - ⊞ P **GO:0010358 : leaf shaping** [5 gene products]
          - ⊞ I GO:0010016 : shoot morphogenesis [174 gene products]
            - ⊞ P GO:0009965 : leaf morphogenesis [128 gene products]
              - ⊞ P **GO:0010358 : leaf shaping** [5 gene products]
        - ⊞ I GO:0048513 : organ development [8801 gene products]
          - ⊞ P GO:0009887 : organ morphogenesis [3816 gene products]
            - ⊞ I GO:0009965 : leaf morphogenesis [128 gene products]
              - ⊞ P **GO:0010358 : leaf shaping** [5 gene products]

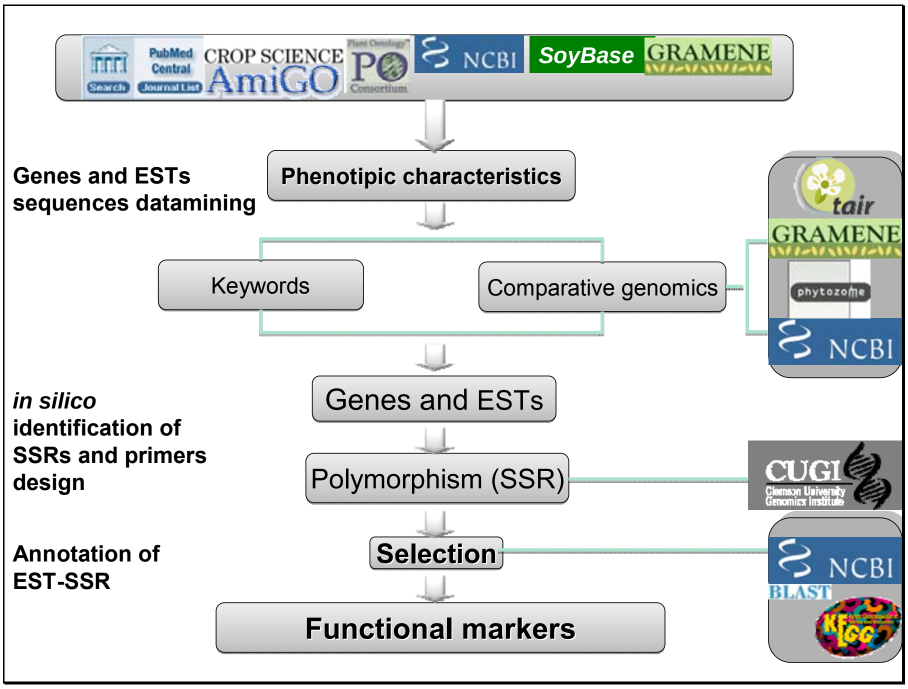
Bioinformátic tools, metabolic pathways



Kyoto Encyclopedia of Genes and Genomes:
http://www.genome.jp/kegg/

Searching for markers in QTLs

- Complex traits – additive effect of genes
- Quantitative traits locus (QTL)
  - Location of gene sequences
  - Potential candidate genes
  - Development of functional markers
- Limits of the QTL interval
  - High degree of resolution, 1 to 2 cM
  - > A 40% change in character



Genes and ESTs sequences datamining

Phenotipic characteristics

Keywords

Comparative genomics

***in silico* identification of SSRs and primers design**

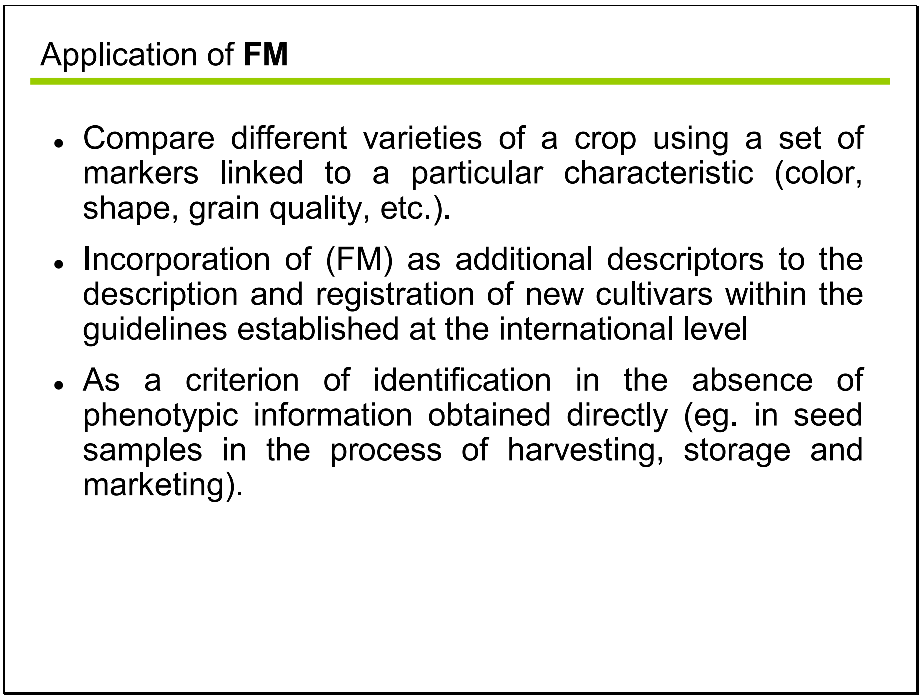
Genes and ESTs

Polymorphism (SSR)

Annotation of EST-SSR

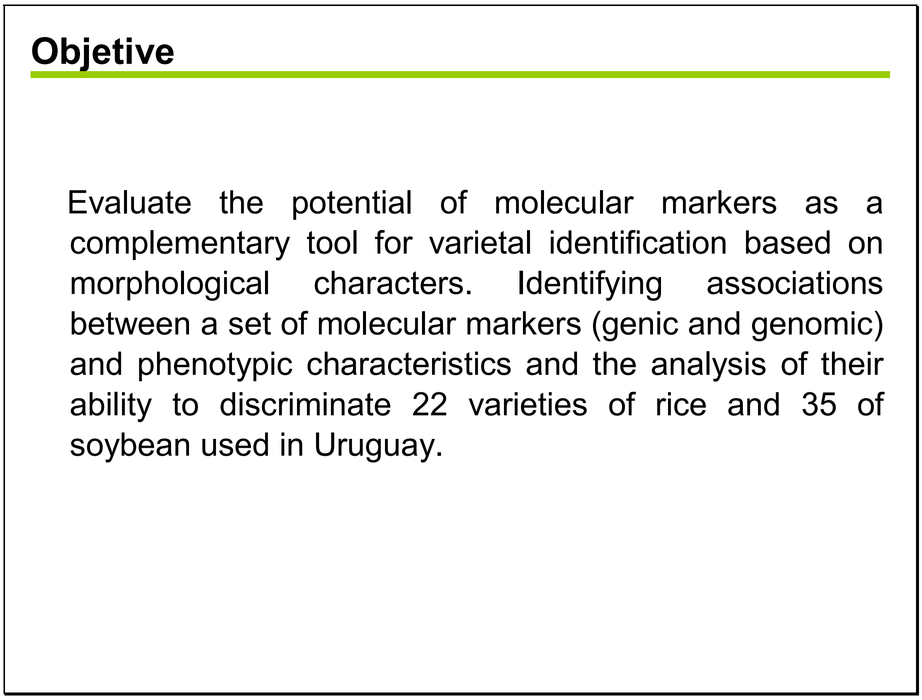
Selection

Functional markers



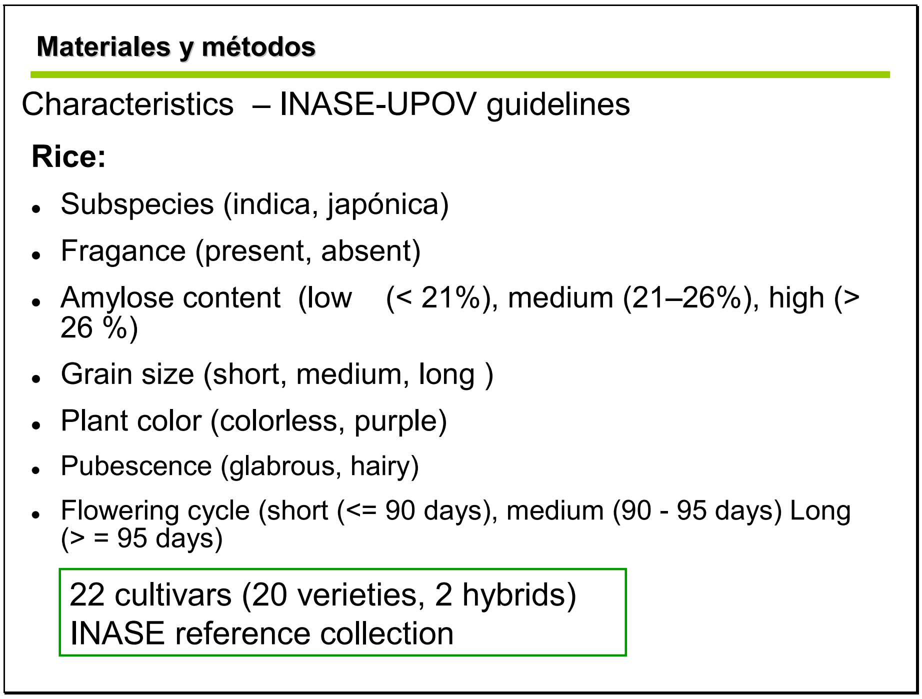
Application of **FM**

- Compare different varieties of a crop using a set of markers linked to a particular characteristic (color, shape, grain quality, etc.).
- Incorporation of (FM) as additional descriptors to the description and registration of new cultivars within the guidelines established at the international level
- As a criterion of identification in the absence of phenotypic information obtained directly (eg. in seed samples in the process of harvesting, storage and marketing).



Objetive

Evaluate the potential of molecular markers as a complementary tool for varietal identification based on morphological characters. Identifying associations between a set of molecular markers (genic and genomic) and phenotypic characteristics and the analysis of their ability to discriminate 22 varieties of rice and 35 of soybean used in Uruguay.



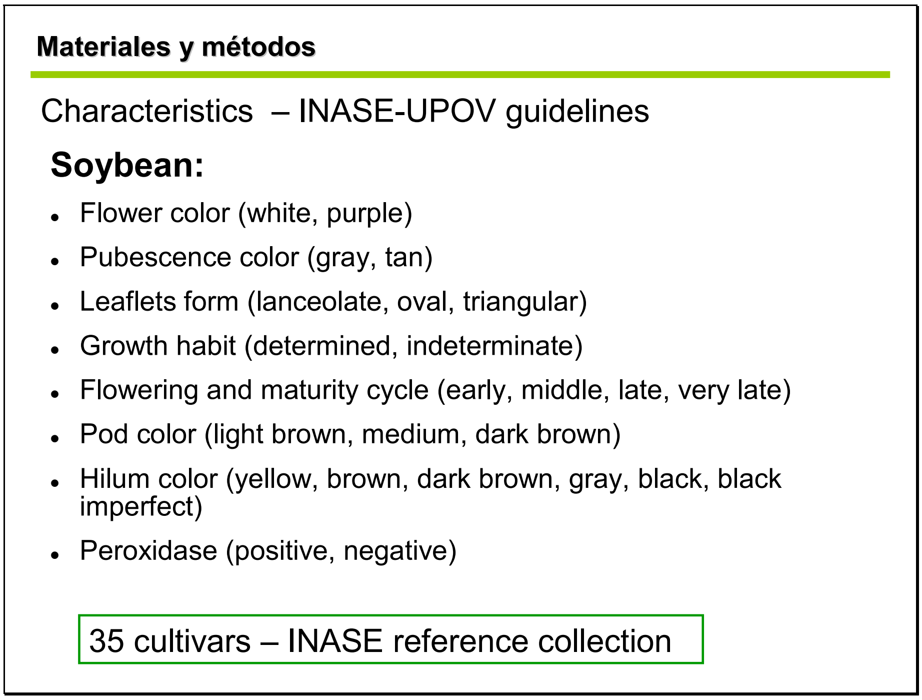
Materiales y métodos

Characteristics – INASE-UPOV guidelines

Rice:

- Subspecies (indica, japónica)
- Fragance (present, absent)
- Amylose content (low (< 21%), medium (21–26%), high (> 26 %)
- Grain size (short, medium, long)
- Plant color (colorless, purple)
- Pubescence (glabrous, hairy)
- Flowering cycle (short (<= 90 days), medium (90 - 95 days) Long (> = 95 days)

22 cultivars (20 verieties, 2 hybrids)
INASE reference collection



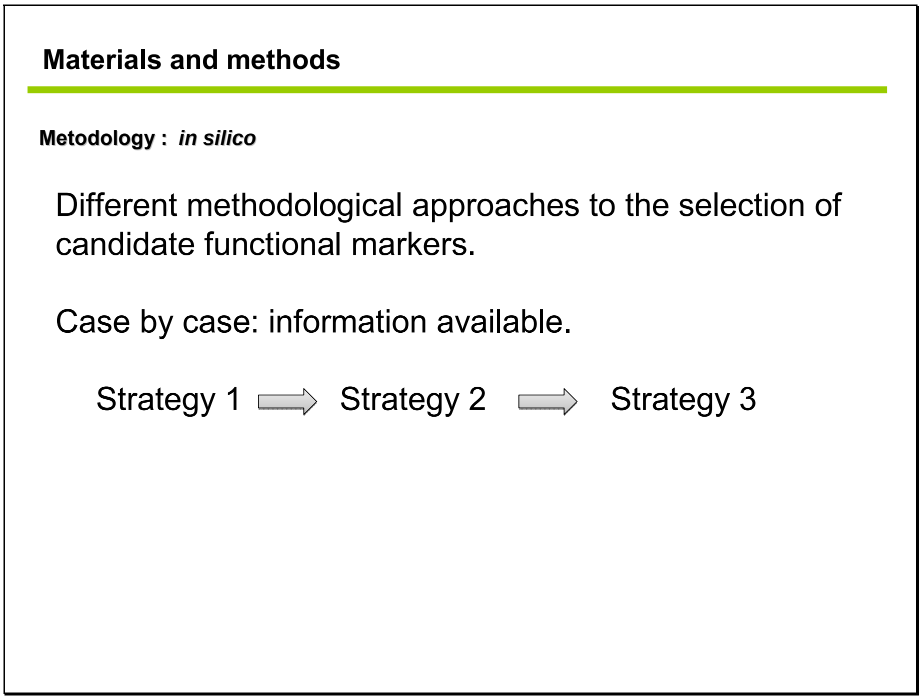
Materiales y métodos

Characteristics – INASE-UPOV guidelines

Soybean:

- Flower color (white, purple)
- Pubescence color (gray, tan)
- Leaflets form (lanceolate, oval, triangular)
- Growth habit (determined, indeterminate)
- Flowering and maturity cycle (early, middle, late, very late)
- Pod color (light brown, medium, dark brown)
- Hilum color (yellow, brown, dark brown, gray, black, black imperfect)
- Peroxidase (positive, negative)

35 cultivars – INASE reference collection



Materials and methods

Metodology : ***in silico***

Different methodological approaches to the selection of candidate functional markers.

Case by case: information available.

Strategy 1 ⇒ Strategy 2 ⇒ Strategy 3

Example - Strategy 1

Soybean: Flower color

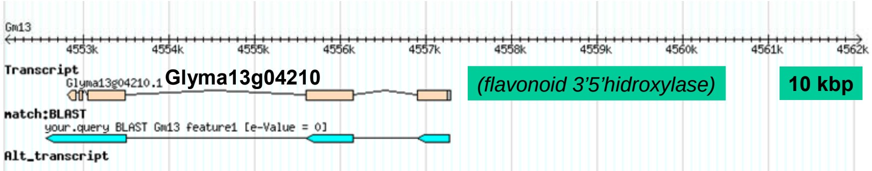
⇩

W1/w1:gmF3’5’H
(*flavonoid 3’5’hidroxylase*)

⇩

Gene specific marker.
Primers:
F-TAG AAA GCA CCC TTC AAC AC-
R-TTT ATG TAG CCA CAG CCA CA-
Expected products:
W1 (526-bp) / w1 (579-bp)

Zabala and Vodkin, 2007 “The mutation is a small (65 bp)insertion of tandem repeats in exon 3 that truncates the translation product prematurely.The presence of this insertion in all-white flower soybean lines ………evidence that the *F3'5'H* gene isolated is likely encoded by the *W1* locus of *Glycine max.”*



Example - Strategy 1

Rice: Amylose content ⇨

Gen: *Waxy* (*Wx1*)
(LOC_Os06g04200)
granule-bound starch synthase

⇩

Phenotypic lass:

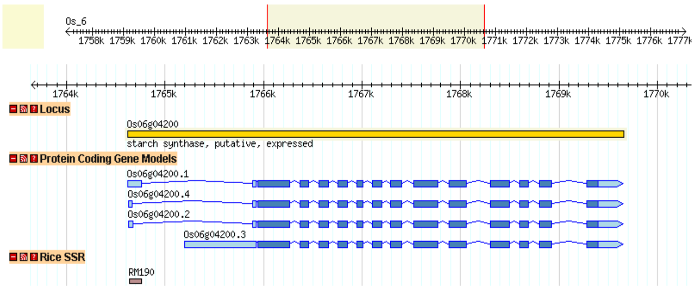
Low: < 21%

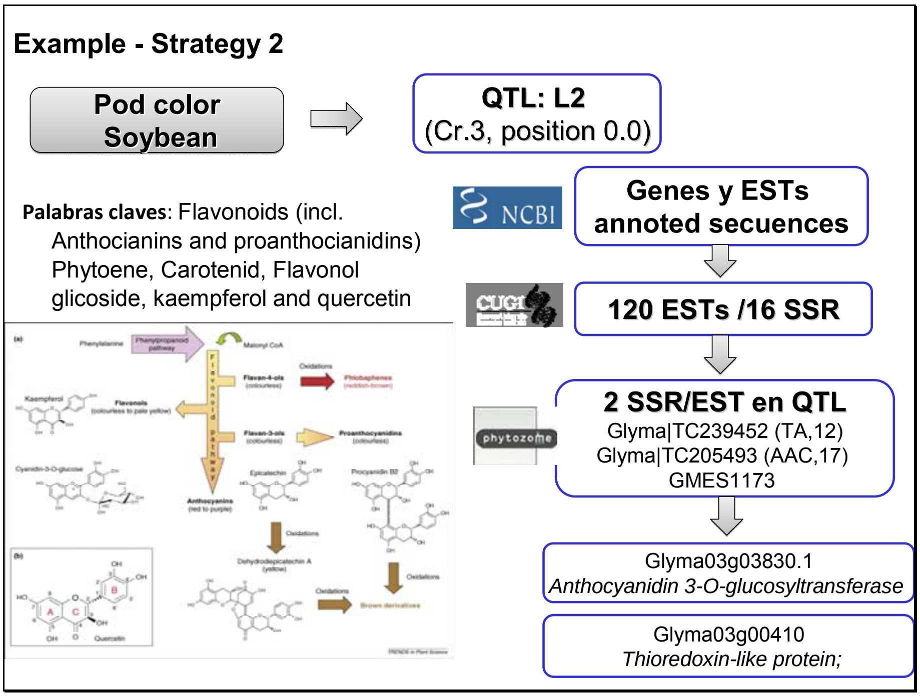
Medium: 21-26 %

High: > 26%

⇩

SSR:RM190 (CTn)
(to 55bp of the end 5´ of the intrón splice site)
Bligh *et al.* 1995





Example - Strategy 2

Pod color Soybean

QTL: L2
(Cr.3, position 0.0)

Palabras claves: Flavonoids (incl. Anthocianins and proanthocianidins) Phytoene, Carotenid, Flavonol glicoside, kaempferol and quercetin

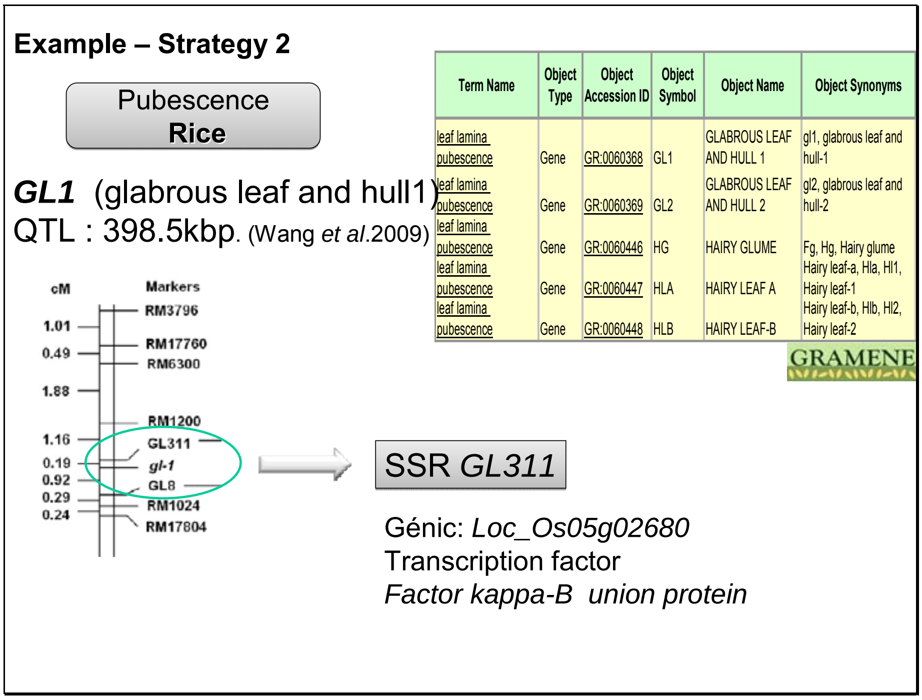
Genes y ESTs annoted secuences

120 ESTs /16 SSR

2 SSR/EST en QTL
Glyma|TC239452 (TA,12)
Glyma|TC205493 (AAC,17)
GMES1173

Glyma03g03830.1
Anthocyanidin 3-O-glucosyltransferase

Glyma03g00410
Thioredoxin-like protein;



Example – Strategy 2

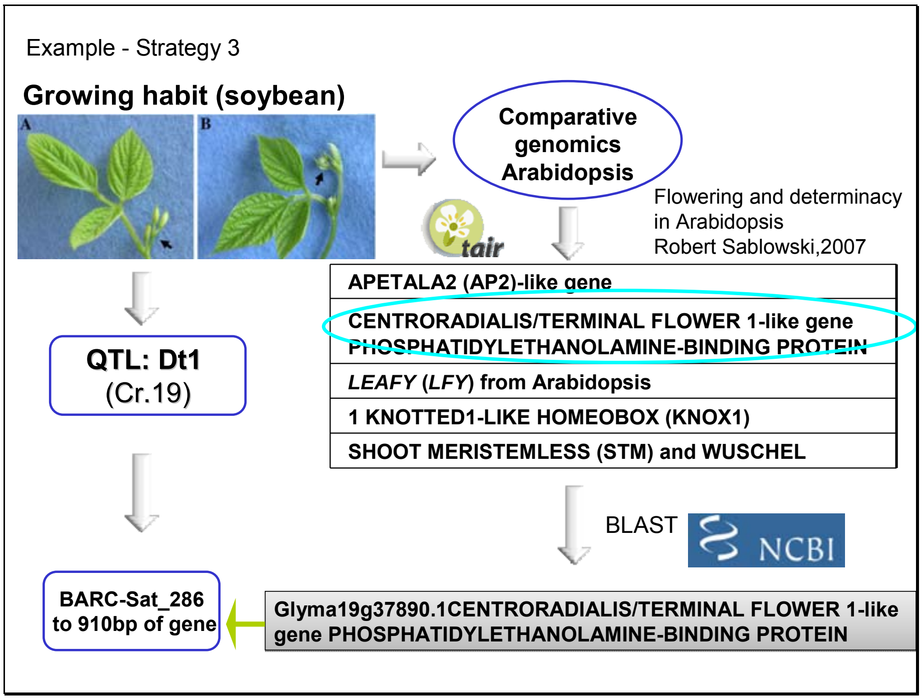
Pubescence
Rice

GL1 (glabrous leaf and hull1)
QTL : 398.5kbp. (Wang *et al.*2009)

Term Name	Object Type	Object Accession ID	Object Symbol	Object Name	Object Synonyms
leaf lamina pubescence	Gene	GR:0060368	GL1	GLABROUS LEAF AND HULL 1	gl1, glabrous leaf and hull-1
leaf lamina pubescence	Gene	GR:0060369	GL2	GLABROUS LEAF AND HULL 2	gl2, glabrous leaf and hull-2
leaf lamina pubescence	Gene	GR:0060446	HG	HAIRY GLUME	Fg, Hg, Hairy glume
leaf lamina pubescence	Gene	GR:0060447	HLA	HAIRY LEAF A	Hairy leaf-a, Hla, Hl1, Hairy leaf-1
leaf lamina pubescence	Gene	GR:0060448	HLB	HAIRY LEAF-B	Hairy leaf-b, Hlb, Hl2, Hairy leaf-2

SSR *GL311*

Génic: *Loc_Os05g02680*
Transcription factor
Factor kappa-B union protein



Example - Strategy 3

Growing habit (soybean)

Comparative genomics Arabidopsis

Flowering and determinacy in Arabidopsis
Robert Sablowski,2007

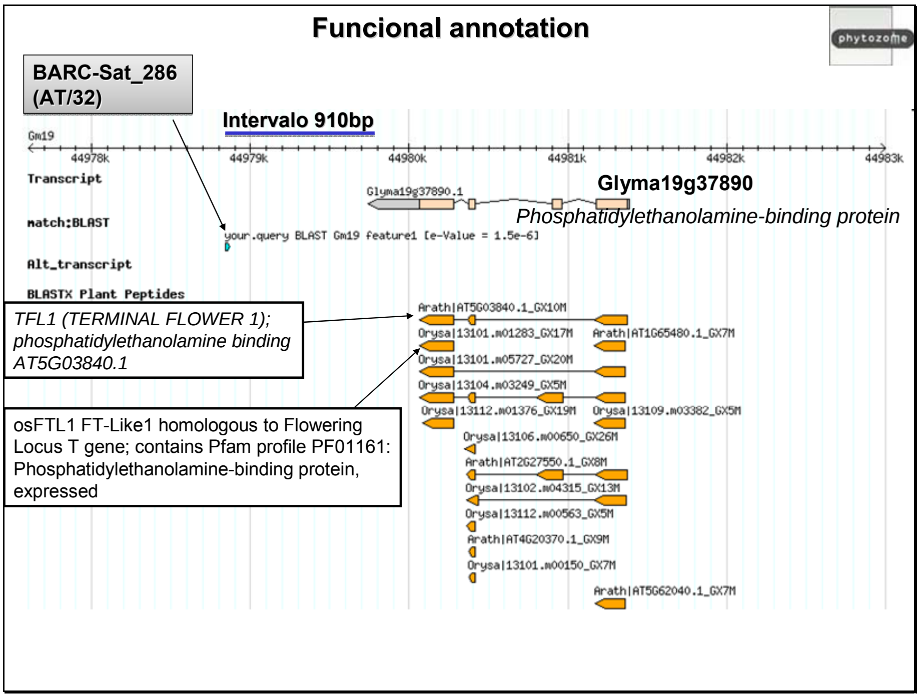
- **APETALA2 (AP2)-like gene**
- **CENTRORADIALIS/TERMINAL FLOWER 1-like gene PHOSPHATIDYLETHANOLAMINE-BINDING PROTEIN**
- ***LEAFY* (*LFY*) from Arabidopsis**
- **1 KNOTTED1-LIKE HOMEOBOX (KNOX1)**
- **SHOOT MERISTEMLESS (STM) and WUSCHEL**

BLAST

QTL: Dt1
(Cr.19)

BARC-Sat_286 to 910bp of gene

Glyma19g37890.1CENTRORADIALIS/TERMINAL FLOWER 1-like gene PHOSPHATIDYLETHANOLAMINE-BINDING PROTEIN



Funcional annotation

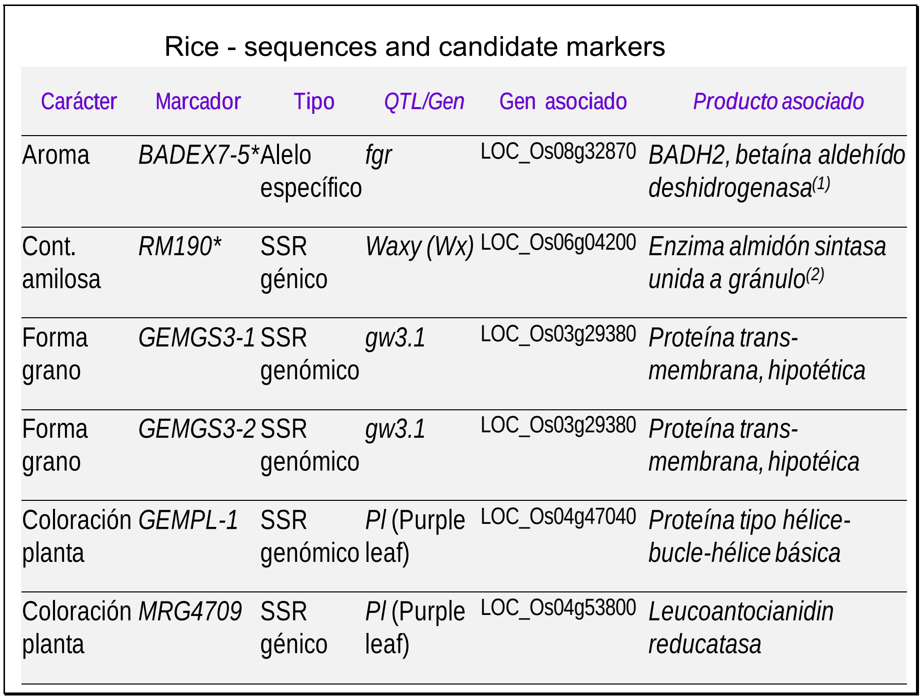
BARC-Sat_286 (AT/32)

Intervalo 910bp

Glyma19g37890
Phosphatidylethanolamine-binding protein

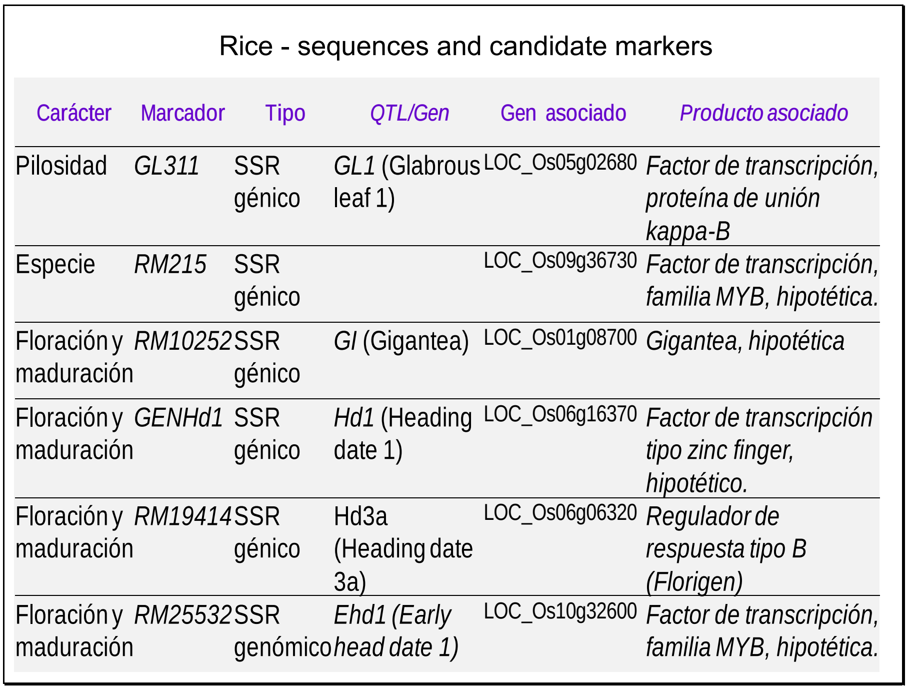
TFL1 (TERMINAL FLOWER 1); phosphatidylethanolamine binding AT5G03840.1

osFTL1 FT-Like1 homologous to Flowering Locus T gene; contains Pfam profile PF01161: Phosphatidylethanolamine-binding protein, expressed



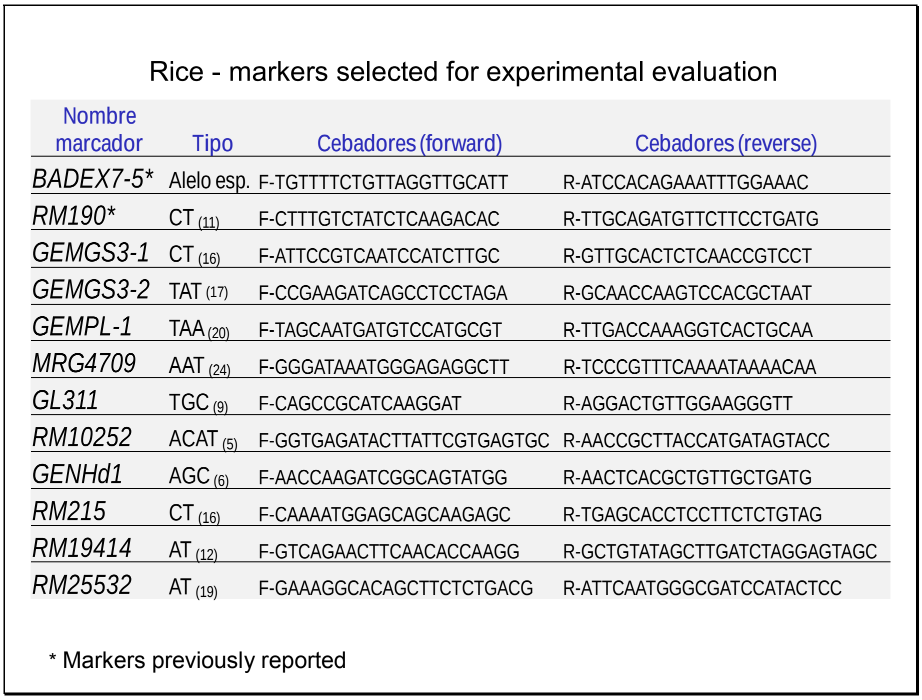
Rice - sequences and candidate markers

Carácter	Marcador	Tipo	*QTL/Gen*	Gen asociado	*Producto asociado*
Aroma	*BADEX7-5**	Alelo específico	*fgr*	LOC_Os08g32870	*BADH2, betaína aldehído deshidrogenasa*(1)
Cont. amilosa	*RM190**	SSR génico	*Waxy (Wx)*	LOC_Os06g04200	*Enzima almidón sintasa unida a gránulo*(2)
Forma grano	*GEMGS3-1*	SSR genómico	*gw3.1*	LOC_Os03g29380	*Proteína trans-membrana, hipotética*
Forma grano	*GEMGS3-2*	SSR genómico	*gw3.1*	LOC_Os03g29380	*Proteína trans-membrana, hipotéica*
Coloración planta	*GEMPL-1*	SSR genómico	*Pl* (Purple leaf)	LOC_Os04g47040	*Proteína tipo hélice-bucle-hélice básica*
Coloración planta	*MRG4709*	SSR génico	*Pl* (Purple leaf)	LOC_Os04g53800	*Leucoantocianidin reducatasa*



Rice - sequences and candidate markers

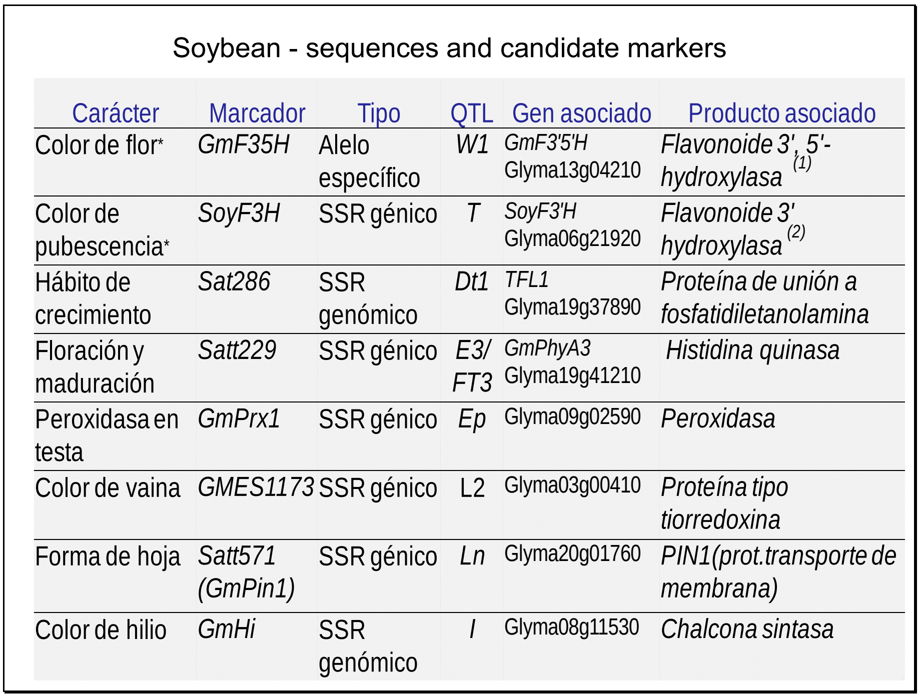
Carácter	Marcador	Tipo	*QTL/Gen*	Gen asociado	*Producto asociado*
Pilosidad	*GL311*	SSR génico	*GL1* (Glabrous leaf 1)	LOC_Os05g02680	*Factor de transcripción, proteína de unión kappa-B*
Especie	*RM215*	SSR génico		LOC_Os09g36730	*Factor de transcripción, familia MYB, hipotética.*
Floración y maduración	*RM10252*	SSR génico	*GI* (Gigantea)	LOC_Os01g08700	*Gigantea, hipotética*
Floración y maduración	*GENHd1*	SSR génico	*Hd1* (Heading date 1)	LOC_Os06g16370	*Factor de transcripción tipo zinc finger, hipotético.*
Floración y maduración	*RM19414*	SSR génico	Hd3a (Heading date 3a)	LOC_Os06g06320	*Regulador de respuesta tipo B (Florigen)*
Floración y maduración	*RM25532*	SSR genómico	*Ehd1 (Early head date 1)*	LOC_Os10g32600	*Factor de transcripción, familia MYB, hipotética.*



Rice - markers selected for experimental evaluation

Nombre marcador	Tipo	Cebadores (forward)	Cebadores (reverse)
*BADEX7-5**	Alelo esp.	F-TGTTTTCTGTTAGGTTGCATT	R-ATCCACAGAAATTTGGAAAC
*RM190**	CT (11)	F-CTTTGTCTATCTCAAGACAC	R-TTGCAGATGTTCTTCCTGATG
GEMGS3-1	CT (16)	F-ATTCCGTCAATCCATCTTGC	R-GTTGCACTCTCAACCGTCCT
GEMGS3-2	TAT (17)	F-CCGAAGATCAGCCTCCTAGA	R-GCAACCAAGTCCACGCTAAT
GEMPL-1	TAA (20)	F-TAGCAATGATGTCCATGCGT	R-TTGACCAAAGGTCACTGCAA
MRG4709	AAT (24)	F-GGGATAAATGGGAGAGGCTT	R-TCCCGTTTCAAAATAAAACAA
GL311	TGC (9)	F-CAGCCGCATCAAGGAT	R-AGGACTGTTGGAAGGGTT
RM10252	ACAT (5)	F-GGTGAGATACTTATTCGTGAGTGC	R-AACCGCTTACCATGATAGTACC
GENHd1	AGC (6)	F-AACCAAGATCGGCAGTATGG	R-AACTCACGCTGTTGCTGATG
RM215	CT (16)	F-CAAAATGGAGCAGCAAGAGC	R-TGAGCACCTCCTTCTCTGTAG
RM19414	AT (12)	F-GTCAGAACTTCAACACCAAGG	R-GCTGTATAGCTTGATCTAGGAGTAGC
RM25532	AT (19)	F-GAAAGGCACAGCTTCTCTGACG	R-ATTCAATGGGCGATCCATACTCC

* Markers previously reported



Soybean - sequences and candidate markers

Carácter	Marcador	Tipo	QTL	Gen asociado	Producto asociado
Color de flor*	*GmF35H*	Alelo específico	*W1*	*GmF3'5'H* Glyma13g04210	*Flavonoide 3', 5'-hydroxylasa* (1)
Color de pubescencia*	*SoyF3H*	SSR génico	*T*	*SoyF3'H* Glyma06g21920	*Flavonoide 3' hydroxylasa* (2)
Hábito de crecimiento	*Sat286*	SSR genómico	*Dt1*	*TFL1* Glyma19g37890	*Proteína de unión a fosfatidiletanolamina*
Floración y maduración	*Satt229*	SSR génico	*E3/ FT3*	*GmPhyA3* Glyma19g41210	*Histidina quinasa*
Peroxidasa en testa	*GmPrx1*	SSR génico	*Ep*	Glyma09g02590	*Peroxidasa*
Color de vaina	*GMES1173*	SSR génico	L2	Glyma03g00410	*Proteína tipo tiorredoxina*
Forma de hoja	*Satt571 (GmPin1)*	SSR génico	*Ln*	Glyma20g01760	*PIN1(prot.transporte de membrana)*
Color de hilio	*GmHi*	SSR genómico	*I*	Glyma08g11530	*Chalcona sintasa*

Soybean - markers selected for experimental evaluation

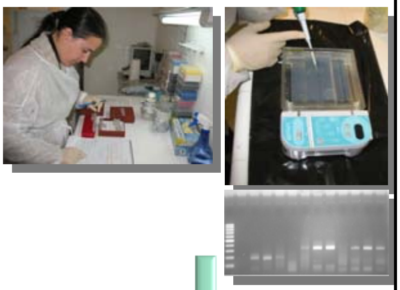
Marcador	Tipo	Cebadores
*GmF35H**	Alelo esp.	F-TAGAAAGCACCCTTCAACAC R-TTTATGTAGCCACAGCCACA
*SoyF3H**	TA (33)	F-GTCATAAAATATCATTATTATTATATCTATTAA R-CACTCCCAAAAGCTTTTAAGTGT
Sat286	AT (32)	F-GCGTTGCTTGCTAAGTAGTGTTTTTAATCCT R-GCGTCTCCCATCATGCAACTTCAATA
Satt229	AAT (22)	F-TGGCAGCACACCTGCTAAGGGAATAAA R-GCGAGGTGGTCTAAAATTATTACCTAT
GmPrx1	TTAA (4)	F-AAAAAGATGTCTTTCAGTTTAGTTTTG R-GCATTACAAGTTTCTGCACCA
GMES1173	AAC (17)	F-TATGGGACATCAAAGCCACA R-CGCACTGCCATATGAAGAGA
GmPin1	ATT (14)	F-TTAGCAATTGGGTAGGGGTG R-CCCTCTTTACGCCAGAAAAA
GmHi	AT (26)	F-CGAATTGGCTGTGTTCTGTG R-GAACCATGTGAGGAAGGCAG

* Markers previously reported

Materials and methods

Genomic DNA extraction

Lab. INASE



Data analysis

PCR

Materials and methods

- Classification analysis (phenotypic classes - molecular information)
- Algorithm “nearest neighbor” (IBK)
- % correct classification

```
0  1  0  1  0  0
1  0  0  0  0  1
0  0  0  0  0  0
1  0  1  0  0  0
0  0  1  0  0  0
0  0  0  0  0  0
0  1  0  1  0  0
1  0  0  0  0  1
0  0  0  0
1  0  1  0
0  0  1  0
0  0  0  0  0  0
```

Matrices 0-1

Waikato Environment for Knowledge Analysi
Weka, a native bird of New
Version 3.6.3
(c) 1999 - 2010
The University of Waikato
Hamilton, New Zealand

(Witten y Frank, 2005).

Kappa Coeficient (Cohen, 1960)
Index of association between values
Phenotypic classes # Molecular data

$$\text{Kappa} = \frac{\Pr(a) - \Pr(e)}{1 - \Pr(e)}$$

Pr(a) = proportion of successes observed
Pr(e) = expected probability of correct answers by chance
k ≤ 0 = random, degree of robustness > k = 1

Rice marker analysis

Nombre marcador	Tipo	Ta.	Nº muestras	Nº alelos	PIC
BADEX7-5	Alelo esp.	43/45	22	2	0.083
RM190	CT (11)	43/45	19	6	0.752
GEMGS3-1	CT (16)	53	20	3	0.368
GEMGS3-2	TAT (17)	53	20	9	0.829
GEMPL-1	TAA (20)	51/53	21	1	0 ⬅
MRG4709	AAT (24)	45	17	5	0.526
GL311	TGC (9)	53	15	6	0.728
RM10252	ACAT (5)	53	17	2	0.374
GENHd1	AGC (6)	51	22	2	0.152
RM215	CT (16)	55	19	5	0.718
RM19414	AT (12)	**	⬅		
RM25532	AT (19)	**	⬅		

Total: 41 alleles, average: 4.1alleles/marker, PIC: 0.45

Soybean marker analysis

Marcador	Tipo	Ta.	Nº. muestras	Nº. Alelos	T. de alelo (bp.)	PIC
GmF35H	Alelo esp.	55	35	2	579, 526	0.375
SoyF3H	TA 33	54	31	5	192, 194, 202, 204, 211	0.529
Sat286	AT 32	54	24	6	164, 166, 168, 176, 178, 180	0.639
Satt229	AAT 22	54	28	4	223, 242, 245, 248	0.402
GmPrx1	TTAA 4	50	29	1	238	0 ⬅
GMES1173	AAC 17	50	31	3	220, 230, 243	0.55
GmPin1	ATT 14	54	34	3	238, 275, 302	0.392
GmHi	AT 26	48	27	1	327	0 ⬅

23 alleles, average 3.13 alleles/marker, PIC: 0.48

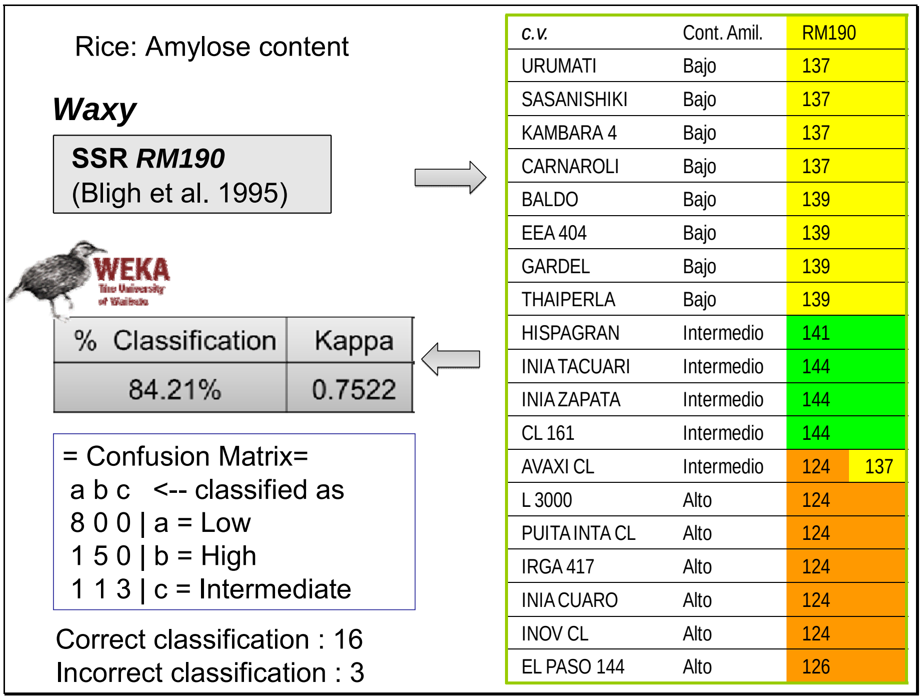
Rice: Pubescence

***GL1* (GLABROUS LEAF AND HULL 1)** WANG D. *et al.* (2009)

***GL311* - SSR/QTL**

Glabrous: Allele 215
Pubescent: Alleles 203, 206, 212, 236

Classification %	Kappa
100%	1



Rice: Amylose content

Waxy

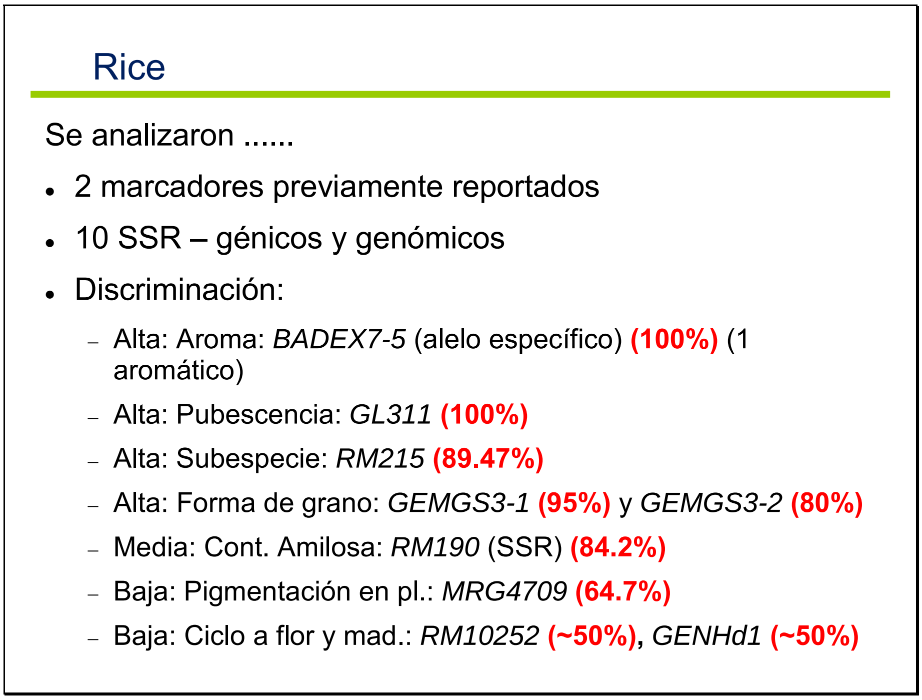
SSR *RM190*
(Bligh et al. 1995)

% Classification	Kappa
84.21%	0.7522

```
= Confusion Matrix=
 a b c   <-- classified as
 8 0 0 | a = Low
 1 5 0 | b = High
 1 1 3 | c = Intermediate
```

Correct classification : 16
Incorrect classification : 3

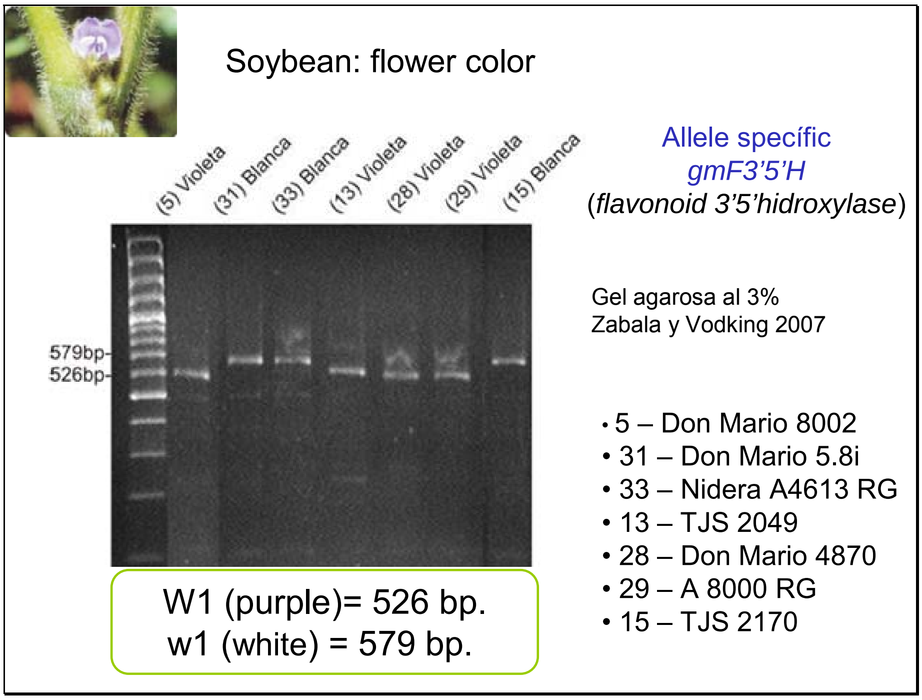
c.v.	Cont. Amil.	RM190	
URUMATI	Bajo	137	
SASANISHIKI	Bajo	137	
KAMBARA 4	Bajo	137	
CARNAROLI	Bajo	137	
BALDO	Bajo	139	
EEA 404	Bajo	139	
GARDEL	Bajo	139	
THAIPERLA	Bajo	139	
HISPAGRAN	Intermedio	141	
INIA TACUARI	Intermedio	144	
INIA ZAPATA	Intermedio	144	
CL 161	Intermedio	144	
AVAXI CL	Intermedio	124	137
L 3000	Alto	124	
PUITA INTA CL	Alto	124	
IRGA 417	Alto	124	
INIA CUARO	Alto	124	
INOV CL	Alto	124	
EL PASO 144	Alto	126	



Rice

Se analizaron ......

- 2 marcadores previamente reportados
- 10 SSR – génicos y genómicos
- Discriminación:
  - Alta: Aroma: *BADEX7-5* (alelo específico) **(100%)** (1 aromático)
  - Alta: Pubescencia: *GL311* **(100%)**
  - Alta: Subespecie: *RM215* **(89.47%)**
  - Alta: Forma de grano: *GEMGS3-1* **(95%)** y *GEMGS3-2* **(80%)**
  - Media: Cont. Amilosa: *RM190* (SSR) **(84.2%)**
  - Baja: Pigmentación en pl.: *MRG4709* **(64.7%)**
  - Baja: Ciclo a flor y mad.: *RM10252* **(~50%)**, *GENHd1* **(~50%)**



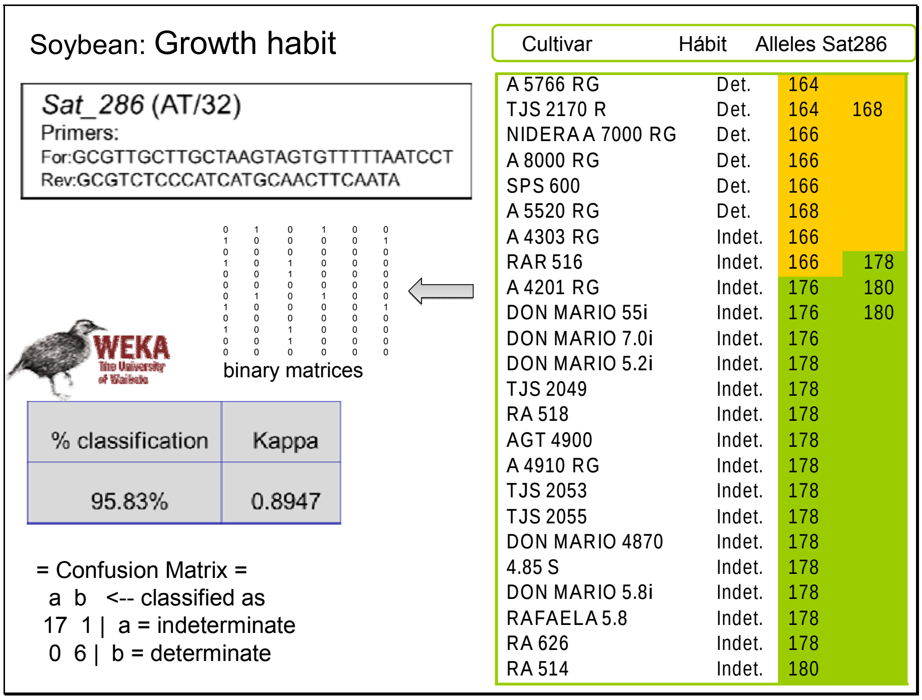
Soybean: flower color

Allele specífic
gmF3'5'H
(*flavonoid 3'5'hidroxylase*)

Gel agarosa al 3%
Zabala y Vodking 2007

- 5 – Don Mario 8002
- 31 – Don Mario 5.8i
- 33 – Nidera A4613 RG
- 13 – TJS 2049
- 28 – Don Mario 4870
- 29 – A 8000 RG
- 15 – TJS 2170

W1 (purple)= 526 bp.
w1 (white) = 579 bp.



Soybean: Growth habit

Sat_286 (AT/32)
Primers:
For:GCGTTGCTTGCTAAGTAGTGTTTTTAATCCT
Rev:GCGTCTCCCATCATGCAACTTCAATA

```
0  1  0  1  0  0
1  0  0  0  0  1
0  0  0  0  0  0
1  0  1  0  0  0
0  0  1  0  0  0
0  0  0  0  0  0
0  1  0  1  0  0
1  0  0  0  0  1
0  0  0  0  0  0
1  0  1  0  0  0
0  0  1  0  0  0
0  0  0  0  0  0
```

binary matrices

% classification	Kappa
95.83%	0.8947

```
= Confusion Matrix =
  a  b   <-- classified as
 17  1 |  a = indeterminate
  0  6 |  b = determinate
```

Cultivar	Hábit	Alleles Sat286	
A 5766 RG	Det.	164	
TJS 2170 R	Det.	164	168
NIDERA A 7000 RG	Det.	166	
A 8000 RG	Det.	166	
SPS 600	Det.	166	
A 5520 RG	Det.	168	
A 4303 RG	Indet.	166	
RAR 516	Indet.	166	178
A 4201 RG	Indet.	176	180
DON MARIO 55i	Indet.	176	180
DON MARIO 7.0i	Indet.	176	
DON MARIO 5.2i	Indet.	178	
TJS 2049	Indet.	178	
RA 518	Indet.	178	
AGT 4900	Indet.	178	
A 4910 RG	Indet.	178	
TJS 2053	Indet.	178	
TJS 2055	Indet.	178	
DON MARIO 4870	Indet.	178	
4.85 S	Indet.	178	
DON MARIO 5.8i	Indet.	178	
RAFAELA 5.8	Indet.	178	
RA 626	Indet.	178	
RA 514	Indet.	180	

Soybean

Were analyzed...

- 2 markers previously reported:
  6 SSR (within or near sequences of interest)
- Ability to discriminate phenotypes:
  - High – Flower color: *gmF35H* (gene specific) **(100%)**
  - High - Growth habit: *Sat286* **(95.83%)**
  - Intermediate – Pubescence color: *SoyF3H* **(80,6%)**
  - Intermediate – Pod color: GMES1173 **(74.19%)**
  - Intermediate – Form of leaflets: Satt571(GmPin1) **(73.5%)**
  - Low – Flowering and maturity: Satt229 **(↓ 50%)**

Discriminatory power of the set of polymorphic markers used for rice and soybeans

	Nº polymorphic markers	**% discrimination**	**Kappa**	**Correctly classified cvs.**
Rice	9	90.91%	0.90	18 / 22
Soybean	6	65.70%	0.64	23 / 35

Discusión

- ↓ PIC soybean (0.45) and rice (0.48).
  - SSR polymorphism gene <genomic SSR
  - ↓ genetic diversity among cvs. used commercial varieties.

Soybeans:
low discriminatory power (65.7%)
↓ number of markers (6 polymorphic)
Narrow genetic base

Rice:
Good discriminatory power (90.9%)
Without discrimination (Puita INTA CL-IRGA 417) and (L3000 - CH crown)
> Number of markers (9 polymorphic)

Discusión

- **Identified some interest associations in Rice and Soybean**
- **Promising results** (development and evaluation of markers with functional value)
- But ....
  They are **not sufficient** to discriminate all genotypes
  The markers do not discriminate 100% of phenotypes

Discusión

To consider:

- Very complex characters (polygenic) (eg. flowering, maturity, form of leaflets, etc.).
- Allelic variations in its class = not fully explain the phenotype, other variations in the gene explain (eg SoyF3H and RM190).
- Indirect searches: less chance of success. Eg. QTLs (≤ 1 to 2 cm)
- Phenotypic data (classes possess variability) to classify complex (eg pigmentation)
- Molecular information must be available

Conclusión

- Molecular markers are no substitute the morphological assessment
- …but they have advantages and applications
  - Additional registration and controller
  - For the evaluation of complex or expensive phenotypic characteristics (resistance, quality, special growing conditions)
  - For test late cycle expression of characteristics (2 to 3 years)
  - Obtain early data
  - Different matrices (seed, harvesting, storage, etc.).
  - Cost effective
  - Laboratories of small and medium scale

GRACIAS

THANKS

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