Proyecto
SAN Nariño

UNIVERSIDAD
NACIONAL
DE COLOMBIA
SEDE BOGOTÁ

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Genetic diversity and population structure of diploid potato genotypes

Deissy Juyó Rojas, María F. Álvarez, Andrés Cortés,
Helena Brochero, Christiane Gebhardt and Teresa Mosquera

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Canadian International Development Agency
Agence canadienne de développement international

IDRC | CRDI
International Development Research Centre
Centre de recherches pour le développement international

Diploid potatoes (*Solanum phureja*)

S. stenotomum

SELECTION

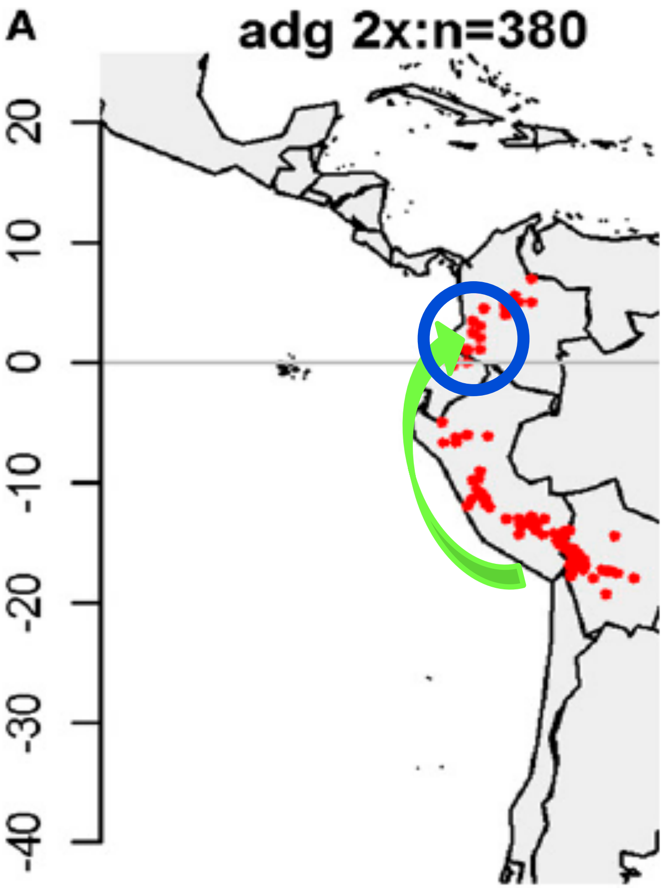
- **Without dormancy**
- **Early maturation**
- **Adaptation to higher temperatures**

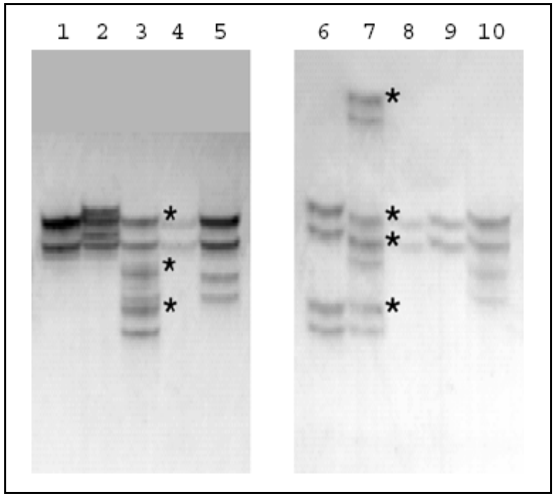
(Hawkes, 1987)

S. phureja

Photo: Rodríguez. (2009)

Distribution, migration of *S. phureja*





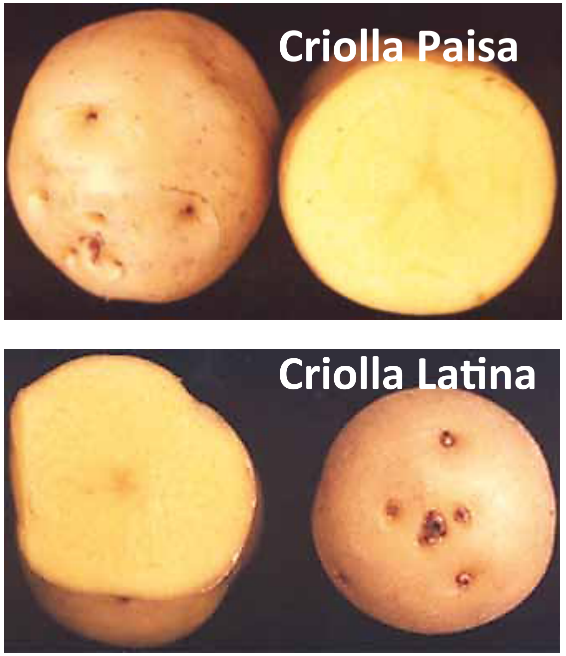
1. (RAPD) - Relatively homogeneous group
2. (SSR) - Differentiated level of ploidy

The Colombo-Ecuatorian mountains are a **diversity center** for this species

Ghislain *et al.*, 1999; Ghislain *et al*., 2006; Spooner *et al.*, 2010

Potato Breeding Program of Universidad Nacional de Colombia

Potato Breeding Program has been working since 1995.



To improve quality tuber for storage and marketing, increasing yield and to look for resistance to pathogens and pest.

Photos: Rodríguez *et al.*, 2009

Variability in *S. phureja* - Colombia

Photo: Angarita 2010

- Shape of corolla, stigma and tuber color.
- Precocity.
- Industrial processing: significant differences in dry matter content, specific gravity and absorption of oils.
- Resistance to pests (*Tecia solanivora*) and diseases (*Phytophthora infestans*)

Escallón and Ramírez, 1997; Martin *et al.,* 2000; Ocampo, 2003; Manrique and Ñustez, 2010

Objective

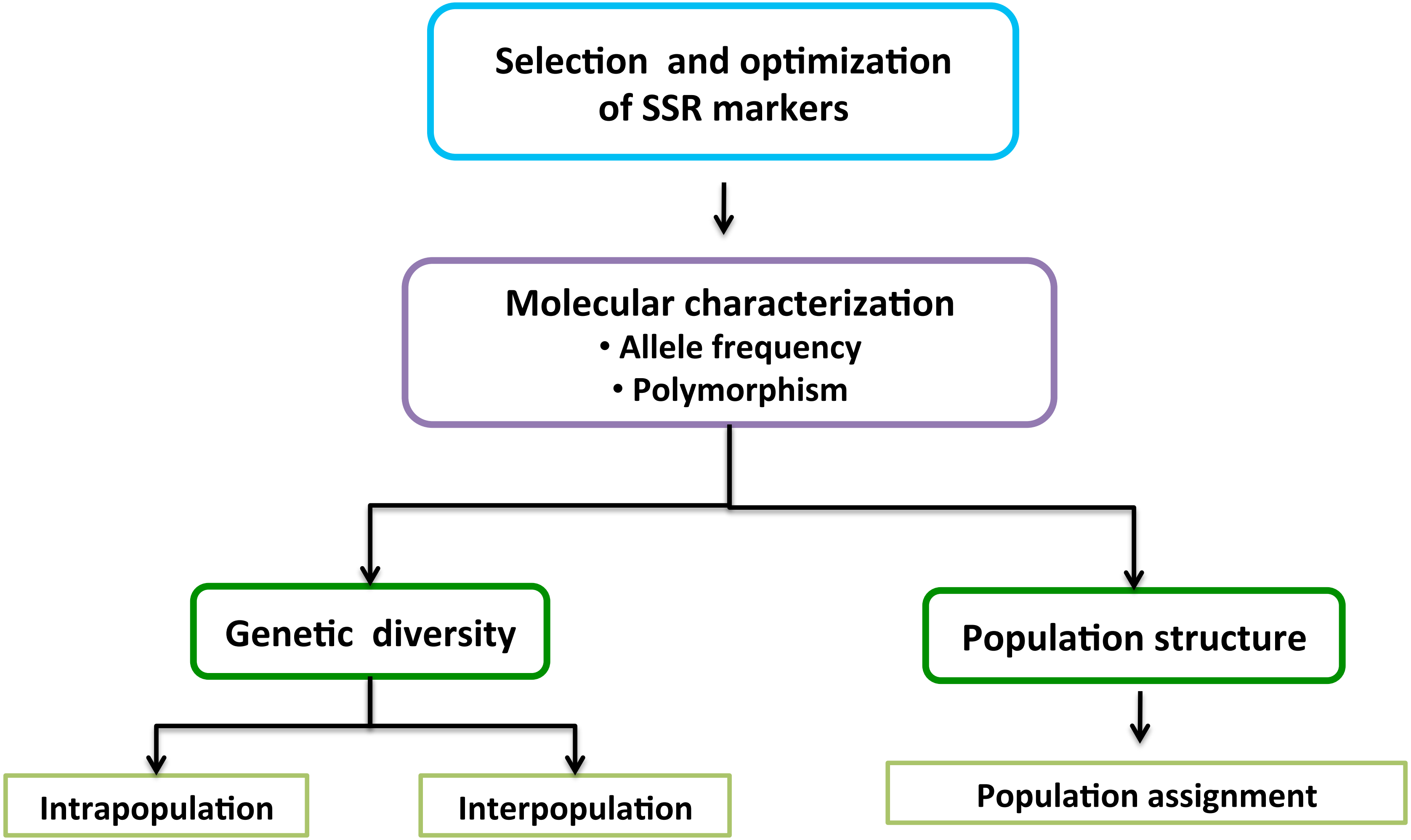
To determine the genetic diversity and population structure of diploid potato genotypes through genetic characterization with SSR markers.

Plant material

	Populations	ID	Genotypes	Origen
Natural	**Colombian Core Collection**	CCC	97	Collected in different regions of Colombia
	Gatersleben Bank	IPK	13	Different countries

Photo: L. E. Rodríguez

Methodology



Selection and optimization of SSR markers

Molecular characterization
- Allele frequency
- Polymorphism

Genetic diversity

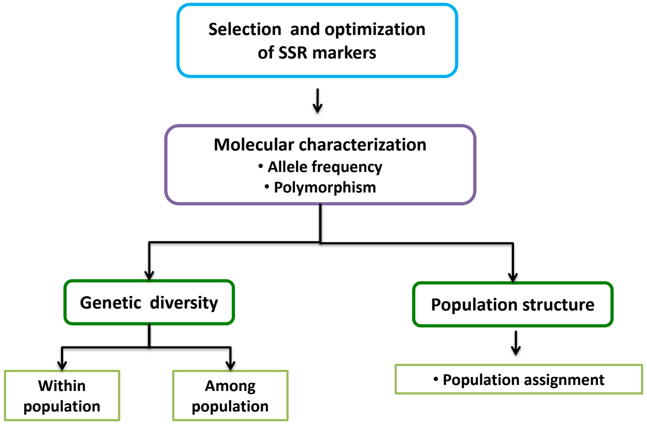
Intrapopulation

Interpopulation

Population structure

Population assignment

Methodology
results and discussion



Selection and optimization of SSR markers

Molecular characterization
- Allele frequency
- Polymorphism

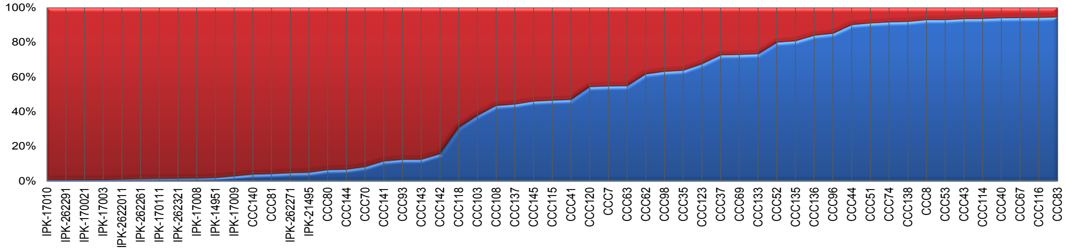
Genetic diversity

Within population

Among population

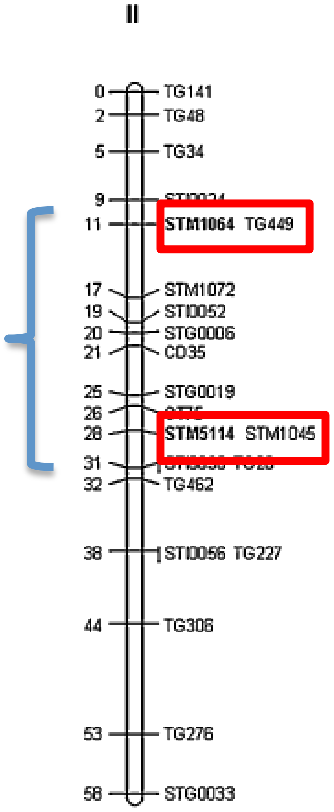
Population structure

- Population assignment



SSR marker selection criteria:

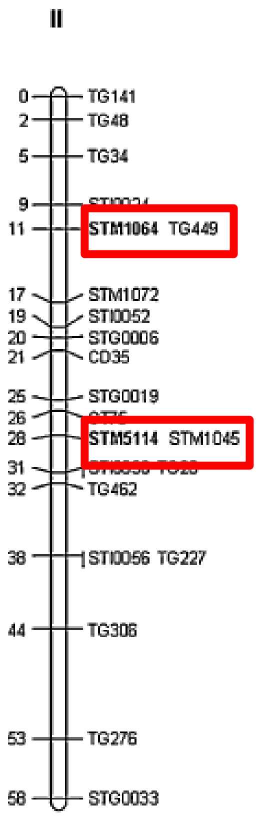
Milbourne *et al*., 1998, Feingold *et al.*, 2005, Ghislain *et al.,* 2006 - 2009.

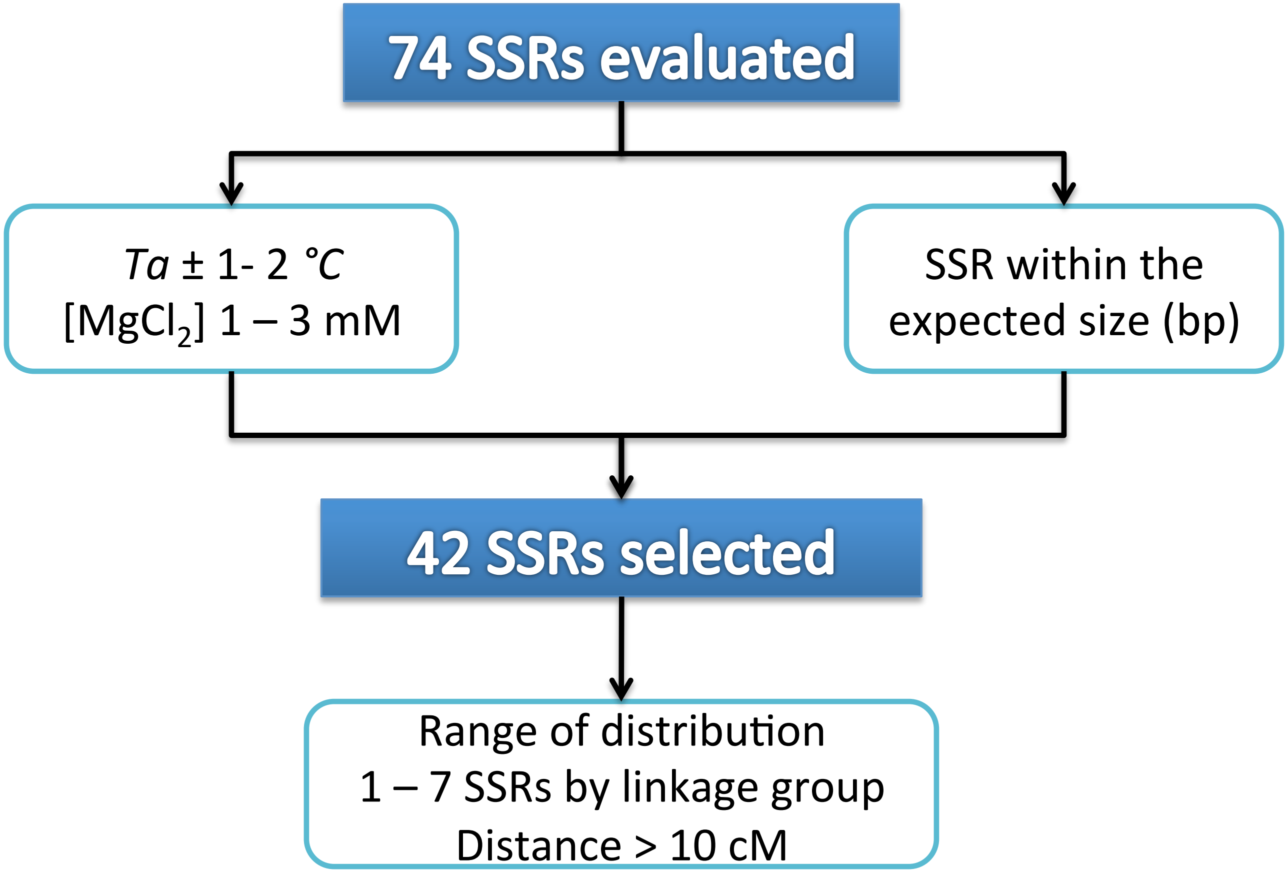


Criteria:

- Distribution in the 12 linkage groups of potato
- Absence of genetic linkage (> 5 cM)
- Optimization based on the concentration of $MgCl_2$, *Ta* (annealing temperature)
- Optimal level of resolution

Selection and optimization of SSR markers





74 SSRs evaluated

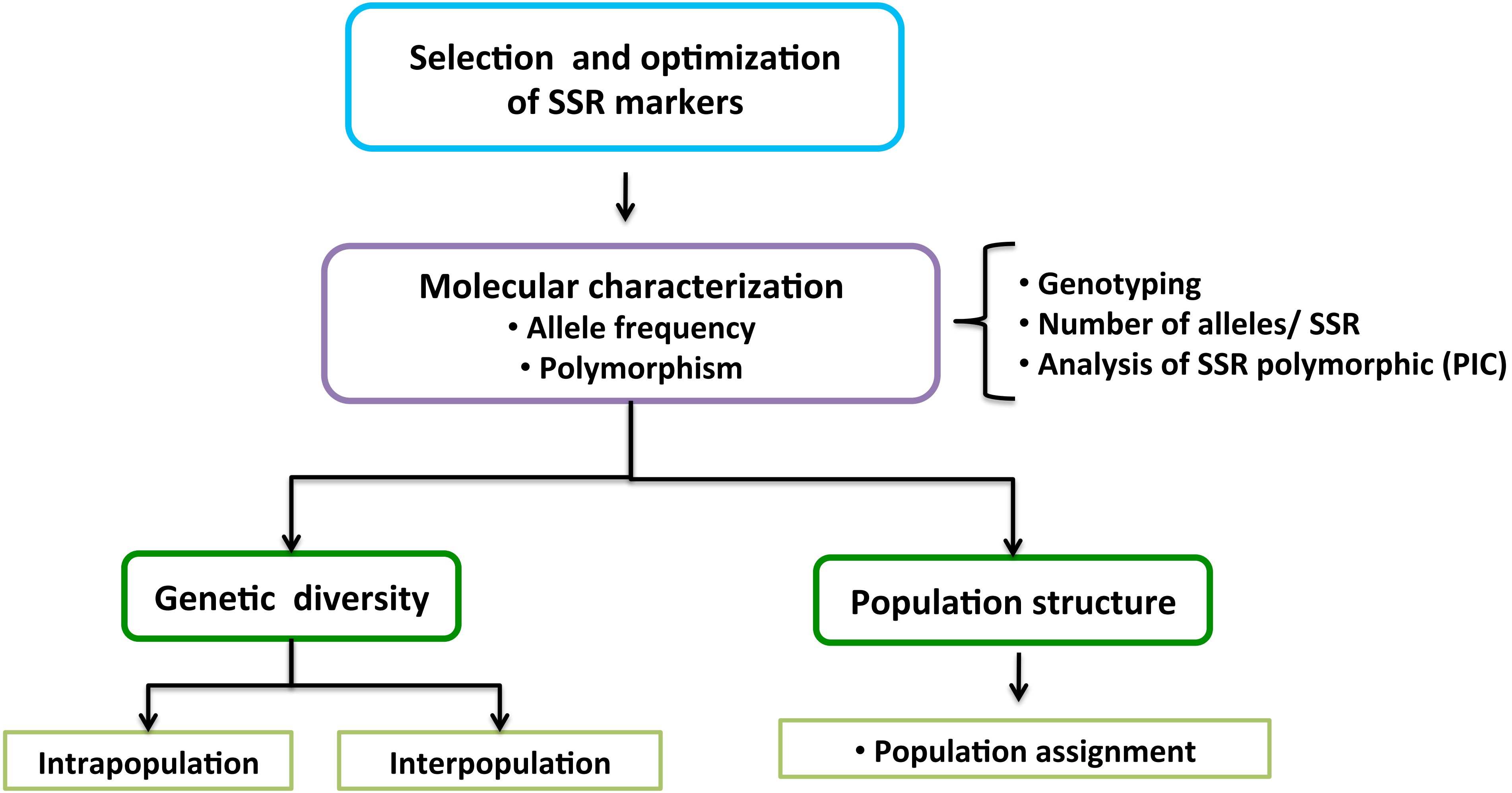
Ta ± 1- 2 °C
$[MgCl_2]$ 1 – 3 mM

SSR within the
expected size (bp)

42 SSRs selected

Range of distribution
1 – 7 SSRs by linkage group
Distance > 10 cM

Methodology



Selection and optimization of SSR markers

Molecular characterization
- Allele frequency
- Polymorphism

- Genotyping
- Number of alleles/ SSR
- Analysis of SSR polymorphic (PIC)

Genetic diversity

Intrapopulation

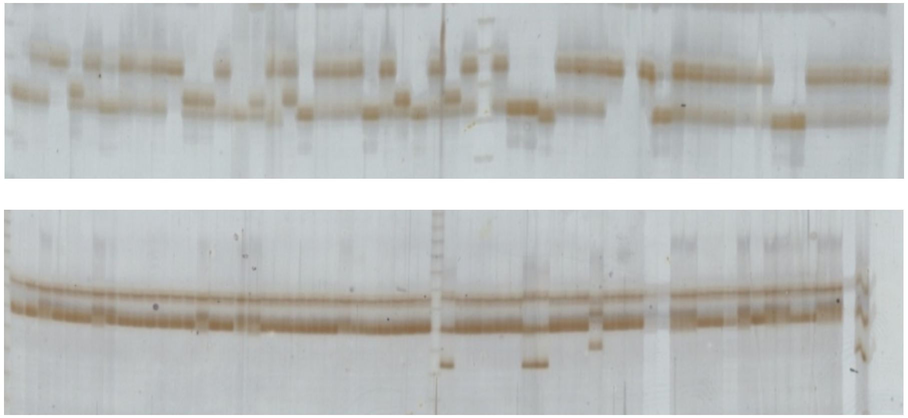
Interpopulation

Population structure

- Population assignment

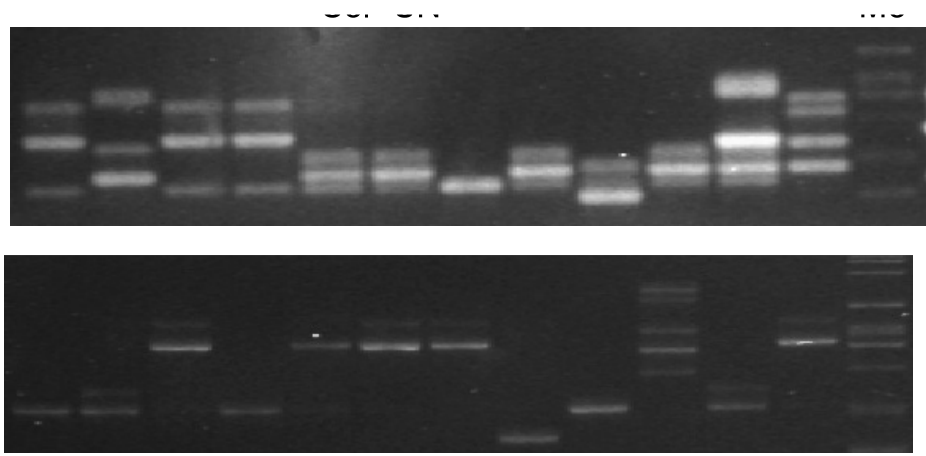
Molecular characterization

SSRs - Poliacrylamide gel 4%



- 23 SSR selected
- Silver stain

SSRs Sepreadex gel



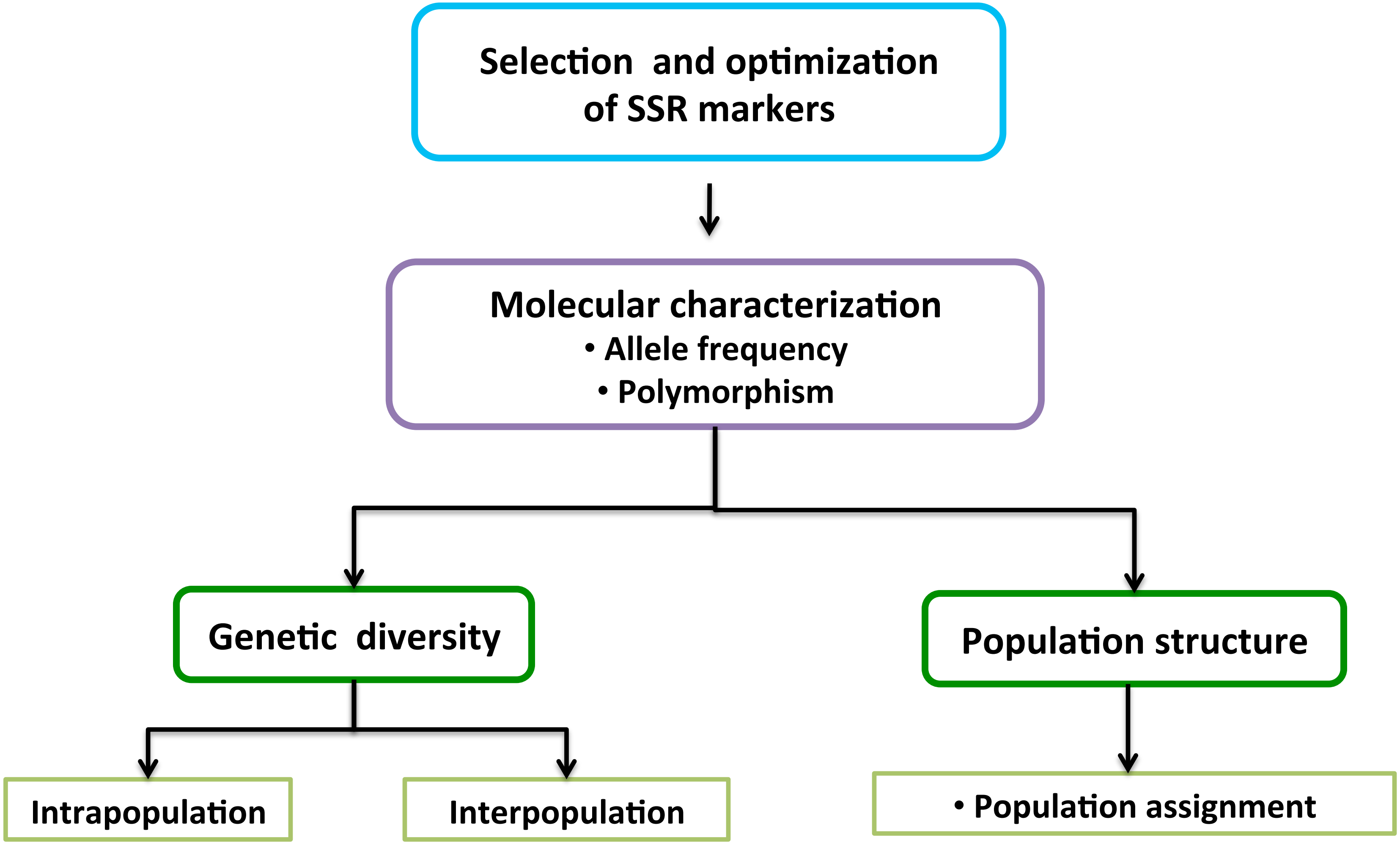
- 19 SSR selected
- Sepreadex gel (EL300, EL600)

Molecular characterization

	Number of alleles	Polymorphic index content (PIC) *
SSRs (range)	2 – 12	0.20 – 0.801
Mean	6	0,458
Total per population	**240**	

* Software Powermarker v.3.25

Methodology



Selection and optimization of SSR markers

Molecular characterization

- Allele frequency
- Polymorphism

Genetic diversity

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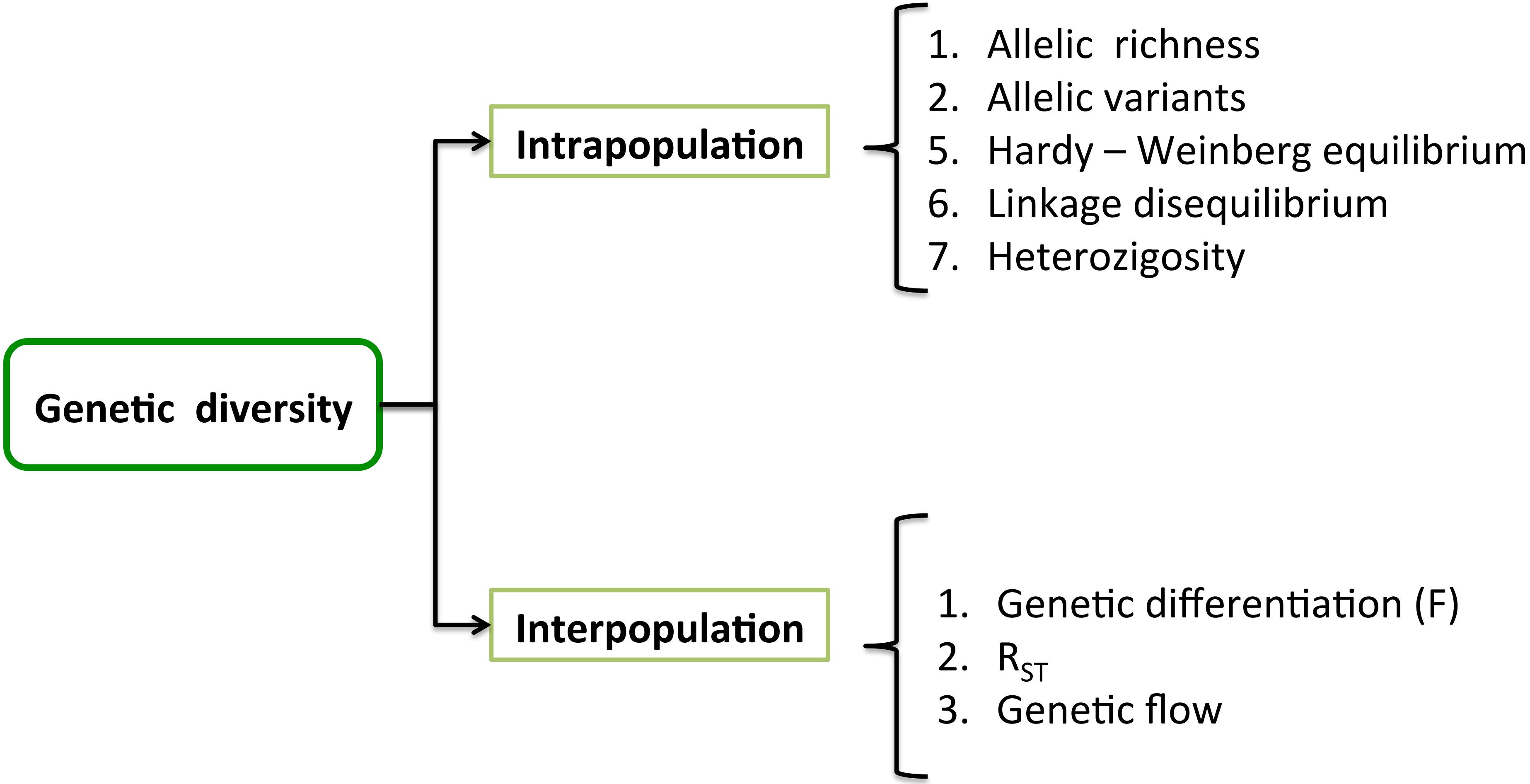
Methodology



Genetic diversity

Intrapopulation

1. Allelic richness
2. Allelic variants
5. Hardy – Weinberg equilibrium
6. Linkage disequilibrium
7. Heterozigosity

Interpopulation

1. Genetic differentiation (F)
2. R_{ST}
3. Genetic flow

Allelic richness and variants

Allelic variants	CCC (N=97)	IPK (N=13)
Number of alleles	1 – 11 (STI023)	1 – 9 (STI021)
Allelic size (bp)	96 - 270	96 - 310
Rare alleles	32 alleles/22 SSR	1 allele / 1 SSR
Private alleles	37 alleles /21 SSR	24 alleles / 16 SSR
Average frequency of null alleles *	0,1152	0,1905

*Maximum likelihood estimation of null allele frequency (Genepop 4.0.10)
EM algorithm (Dempster, Laird and Rubin, 1977) – distinguishes between apparent homozygous and heterozygous for different alleles.

Hardy-Weinberg equilibrium

H0= random union of gametes
H1= heterozygote excess

→

Dememorization number = 1000
Number of batches = 100
Number of iterations per batch = 1000

Exact HW test

P- value (locus/populations)

Marker	CCC
	F_{IS}
StwaX2	-0.344*
STM0025	-0.298*
STI054	-0.959*

Global HW test

Total *P- value* (populations)

Marker	CCC	IPK
Total test Global	P = 1.000 SD = 0.000	P = 1.000 SD = 0.000

GENEPOP

Linkage Desequilibrium (LD)

Test association between diploid genotypes at two loci → Ho: "*Genotypes at one locus are independent from genotypes at the other locus*"

Populations	CCC	IPK
Pairs of markers in LD	64 (7%) P = 0.0000-0,0009	6 (0,66%) P = 0,0000-0,00013
Common markers in LD	(i.e STGBSS, StwaX2, STG0018, STM1104, STM0025)	

***P-value* :* Analyzed after Bonferroni correction for multiple comparisons problems (P < 0,0012)**

Results - Heterozigosity

Populations	H_O	H_S	H_T	D_{ST}	G_{ST}
Natural	0.424	0.555	0.582	0.026	0.045

Ho = Observed heterozygosity
Hs = Expected heterozygosity
H_T = Total heterozygosity

D_{ST} = Gene diversity among populations
G_{ST} = Differentiation between populations on several loci

Diversity - discussion

- High heterozygosity (Hs-diversity) has been maintained despite the artificial selection.

H_s

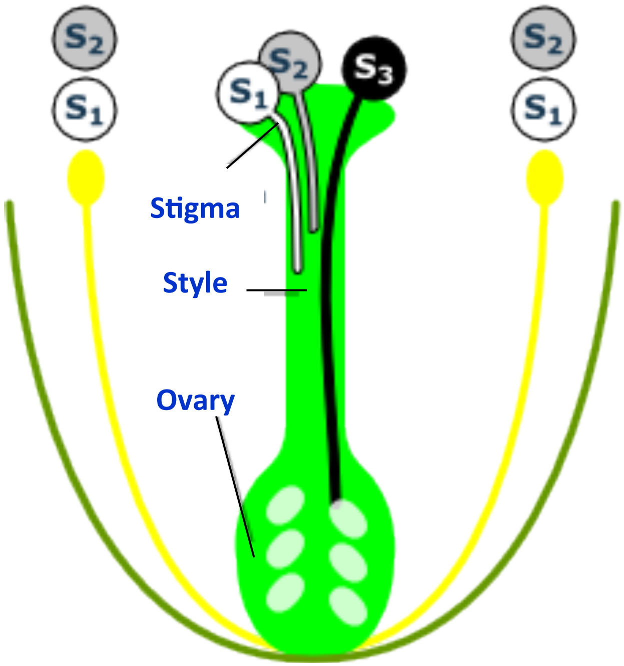
Diploid genotipes from Colombia	**(0.55)**
S. tuberosum group tuberosum	(0.46 – 0.52)
S. tubresosum group andigenum	(0.88)

- High intervarietal hybridization (outcrossing conditions).

Heterozigosity - discussion

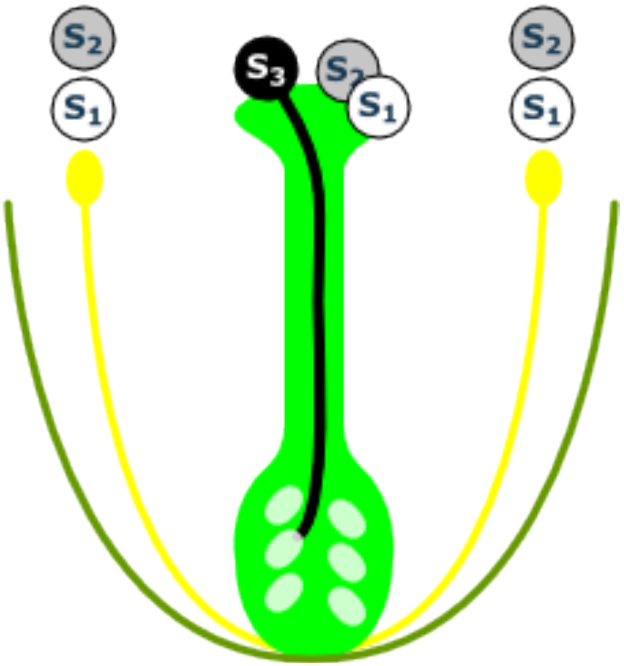
- Diploid potato species have a strong self-incompatibility due to the presence of the *S* allele rejection system, that inhibit fertilization when male and female gametes present the same genetic constitution (Hawkes 1987).

A. Gametofitic



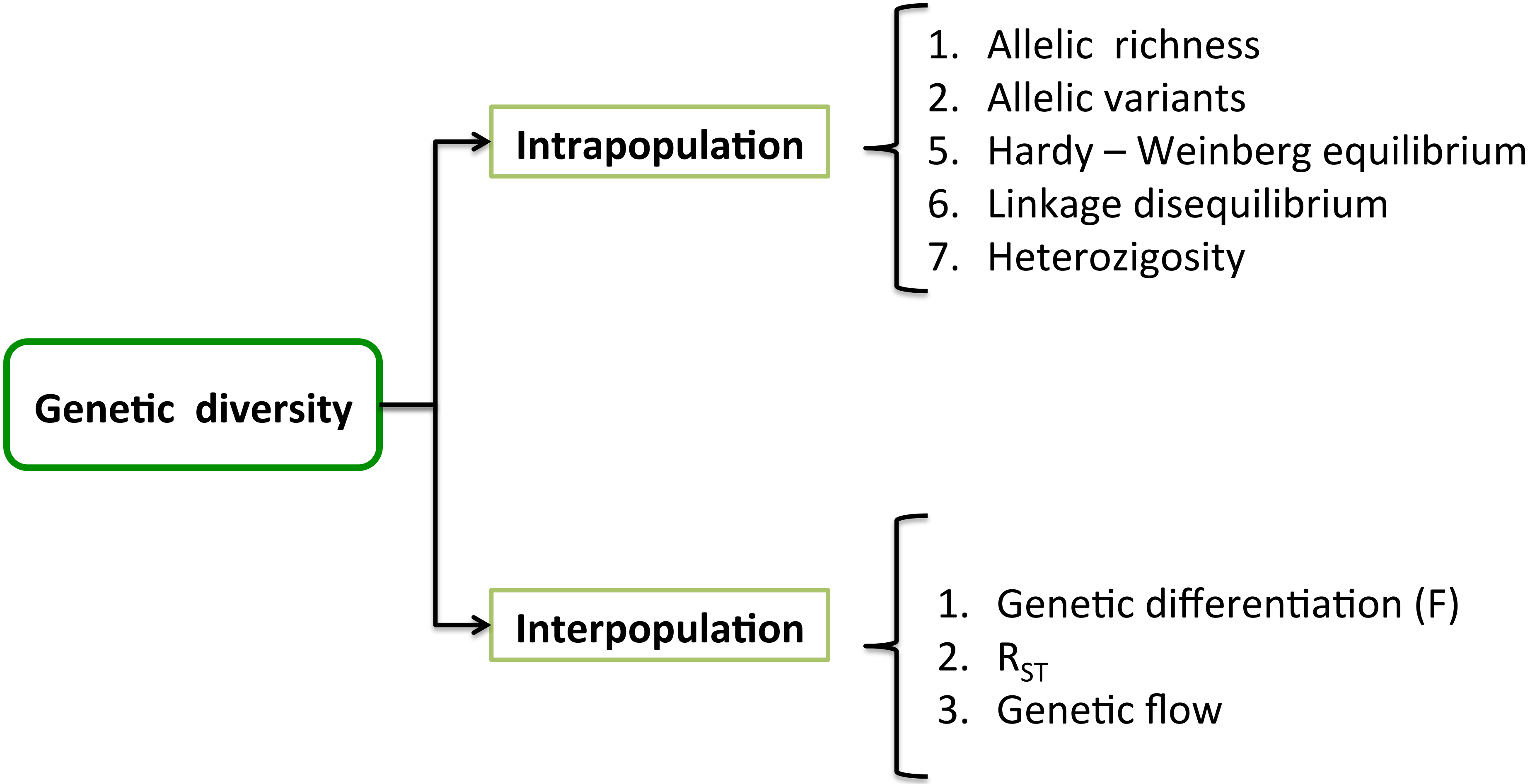
plant S_1S_2

B. Sporofitic



plant S_1S_2

Methodology



Genetic diversity

- **Intrapopulation**
  1. Allelic richness
  2. Allelic variants
  5. Hardy – Weinberg equilibrium
  6. Linkage disequilibrium
  7. Heterozigosity
- **Interpopulation**
  1. Genetic differentiation (F)
  2. R_{ST}
  3. Genetic flow


Arlequin ver 3.11

(c) Laurent Excoffier 1998-2007

Coefficients of genetic differentiation

Populations	F_{IS}	F_{ST}	F_{IT}
Natural	0.17115*	0.09636*	0.25103*

** P- value : 0.00000*

F_{IS}: Inbreeding coefficient within populations ($F_{IS} < 0$ indicate heterozygote excess)
F_{ST}: Coefficient of genetic differentiation between populations
F_{IT}: Total inbreeding coefficient

Rst and Genetic flow

(Slatkin, 1995)

$$M_R = \frac{d_s - 1}{4d_s}\left(\frac{1}{R_{ST}} - 1\right).$$

M= *Nm* = Genetic flow

Estimating migration rates

Population 1	Population 2	R_{ST}	*Nm***
CCC	IPK	0.23635*	1.61554

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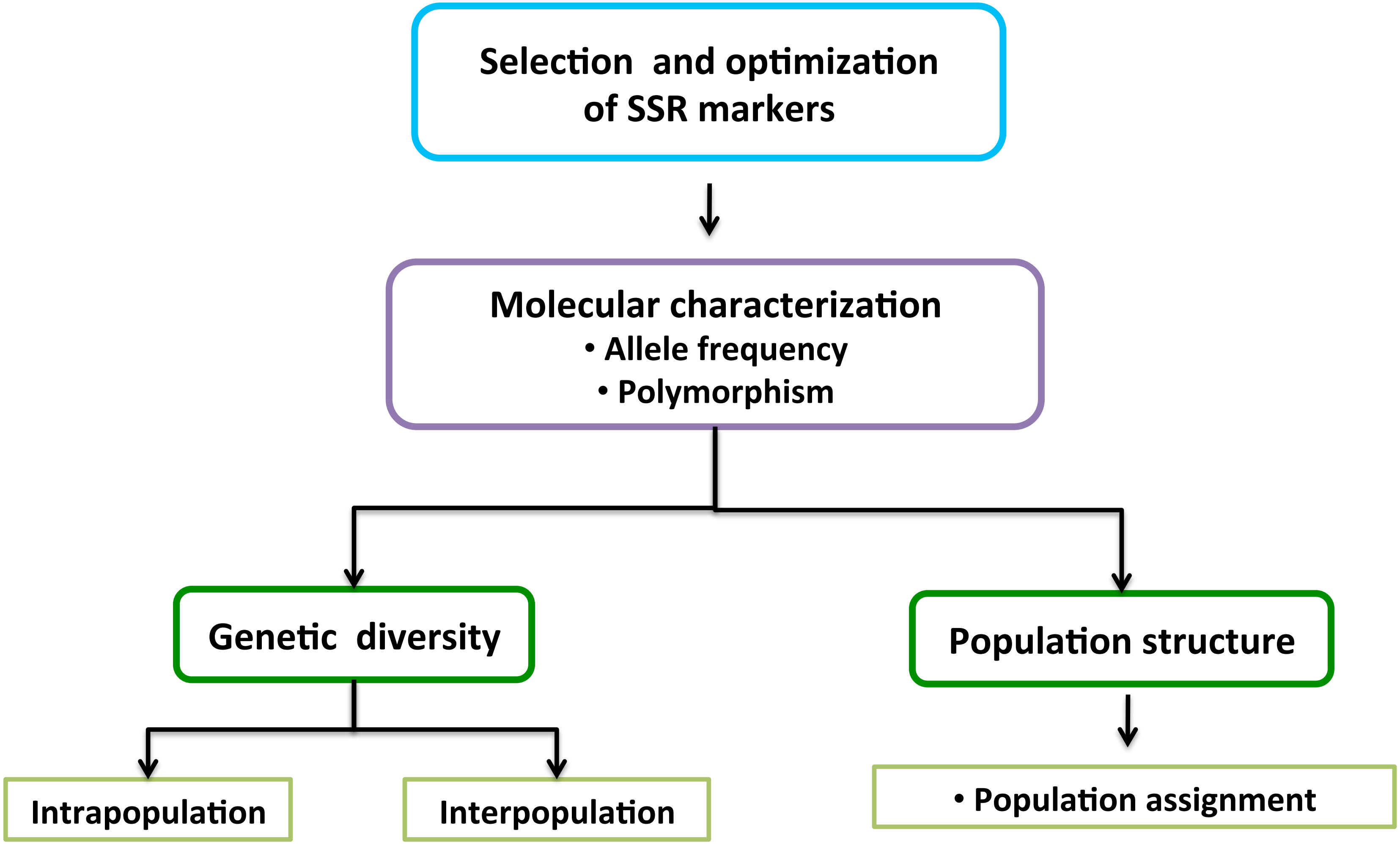
* Significance values ($P \leq 0{,}05$)

** Genetic flow (Nm=M) calculate from Rst

Methodology



Selection and optimization of SSR markers

Molecular characterization
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- Polymorphism

Genetic diversity

Intrapopulation

Interpopulation

Population structure

- Population assignment

Structure

Population structure

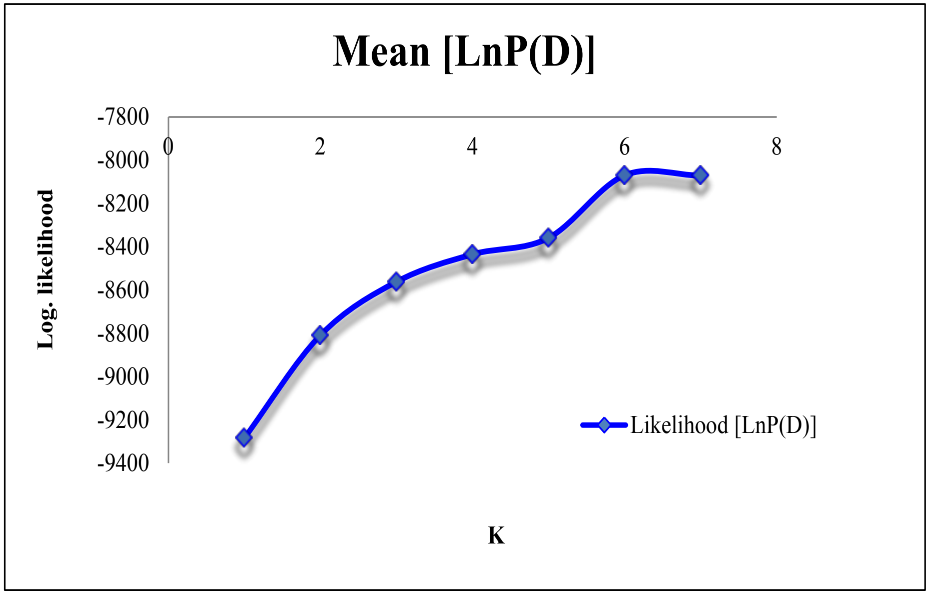
Model-based clustering method
(Bayesian approach)

- **42 SSR**

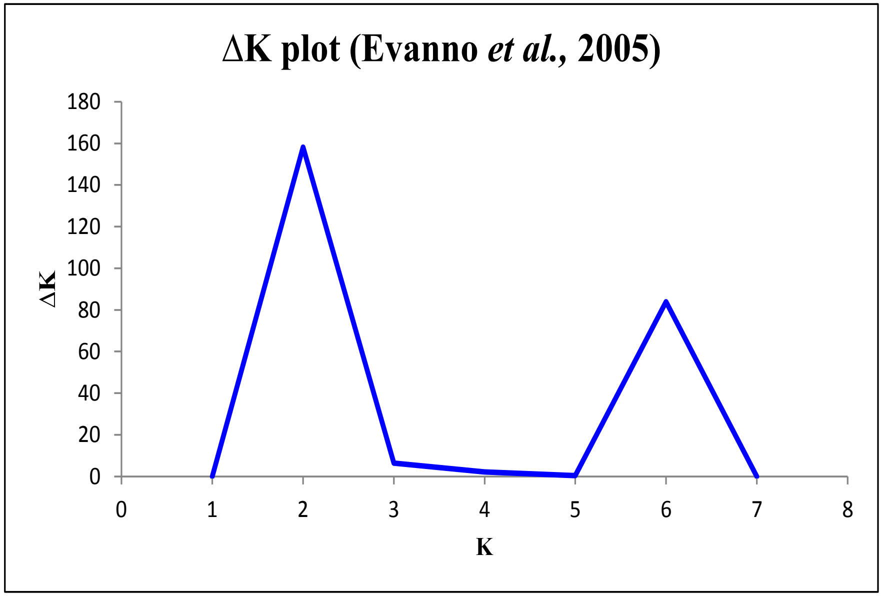
- **Use Admixture Model**
- **Fst = 0.01 and 0.096**

- Number of MCMC Reps after Burning: 300,000
- Length of Burning Period: 30,000
- Number of subgroups—K—ranging from two to ten with ten independent repeats

Likelihood of population assignment



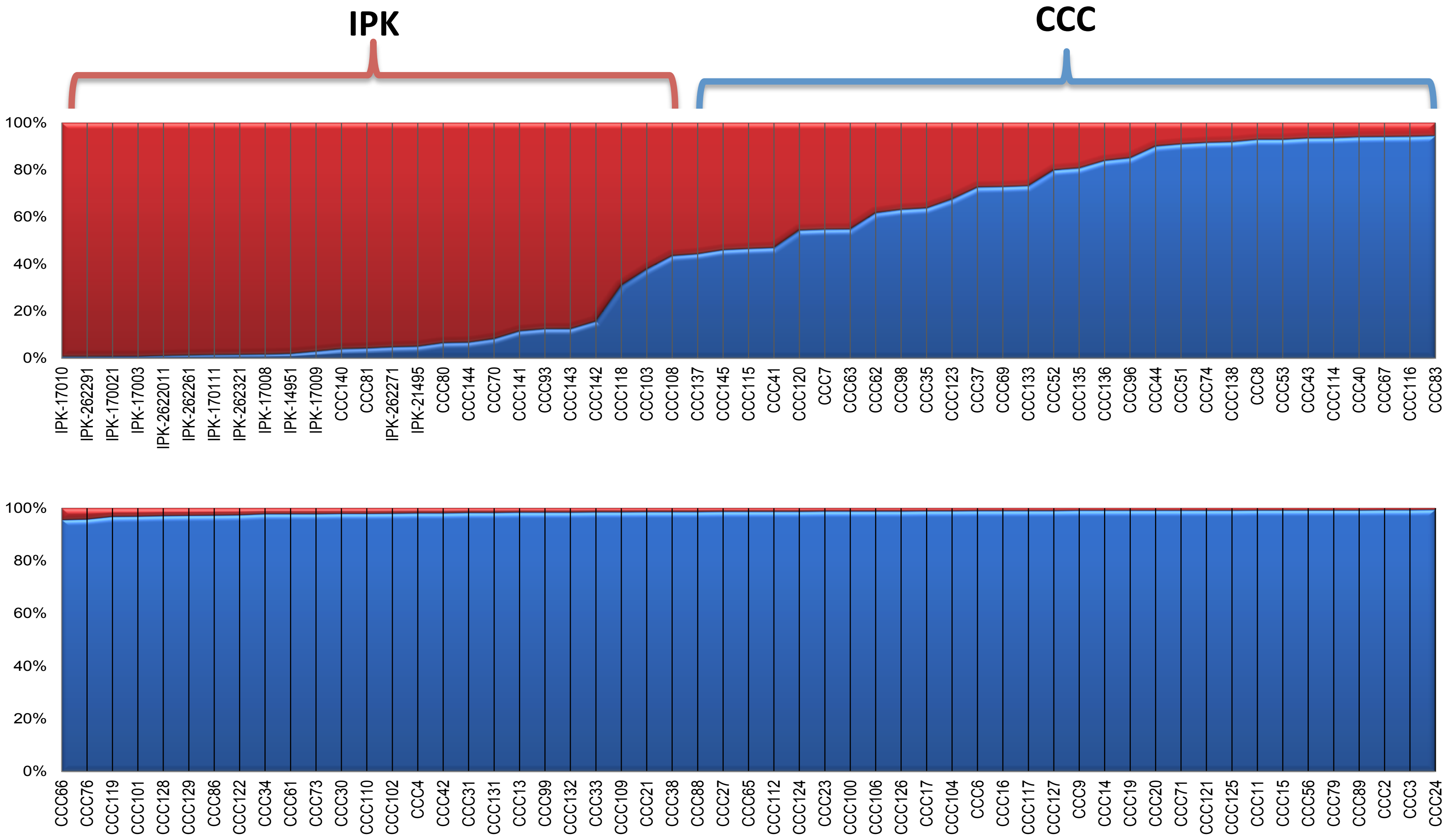
Mean [LnP(D)]



ΔK plot (Evanno *et al.*, 2005)

- The results of *Structure* showed a **continuous increase of the goodness** of fit, Ln[P(D)], versus the number of groups *K*
- **No population structure was found between initial population**
- The Δ statistical test using the STRUCTURE-SUM program showed that K = 2 was optimal in this analysis

True underlying K



STRUCTURE solution. Bar plot of individual potato cultivars generated by STRUCTURE 2.3.2.1 using the admixture model with independent allele frequencies. Groups are represented by colours, as indicated in the legend. Each column (110 in total) represents a cultivar its genotype and is partitioned into segments indicating its likely genetic origin

Conclusion

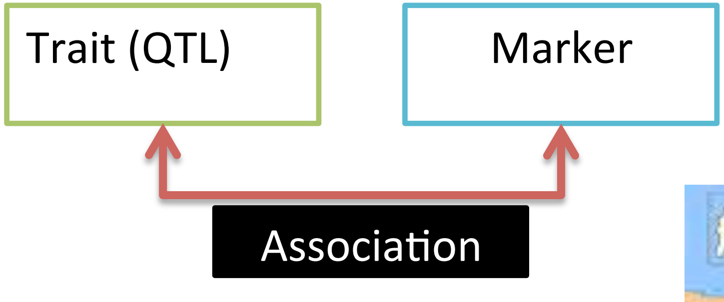
The group of selected SSRs allowed the genetic characterization of the population of interest.

Our results indicated that the population of diploids genotypes from germplasm bank of Colombia has high genetic diversity, possibly due to the high gene flow and the differentiation among individuals within populations.

The lack of population structure (CCC) found in this study allows to use this population for genetic association studies.

Perspectives

1. Association mapping



Trait (QTL)

Marker

Association

2. Design new strategies to collect genotypes to increase genetic diversity in the collection

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Ministerio de Agricultura y Desarrollo Rural

División de Investigación Bogotá
Universidad Nacional de Colombia

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