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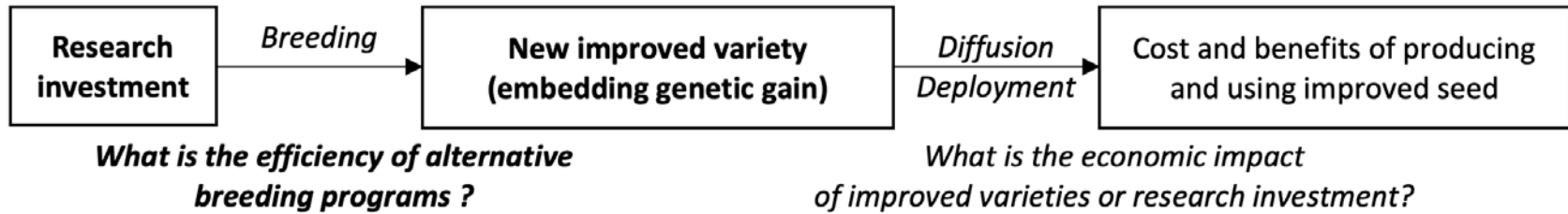
Evaluation of economic efficiency of adaptive breeding strategies for Maritime Pine

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Introduction



- Efficiency of breeding strategy : for a given research investment, what is the impact of various breeding program on the genetic improvement (and possibly over genetic variables)
- A question that is different and complementary to the analysis of the economic impact of improved varieties of research investment (cf. survey by Chang et al. 2019)



Objectives

- Within B4EST :
 - Using modelling, what is the efficiency of new breeding programs compared to the current breeding programs ? Particular attention paid to breeding program based on genomic selection or the use of molecular markers.
 - Three different applications to Maritime Pine, Poplar and Norway Spruce.
- Today, a pragmatic presentation of :
 - Application to Maritime Pine. Jan.-Sept. 2020. All stages completed (leading to deliverable 5.2.), but additional simulation to be made before a publication.
 - Application to Poplar. May-Sept. 2020. Only the first stages completed or initiated.



Definitions and the general principle of the comparison

- A breeding program is based on multiple **basic operations** : crossing parents, growing progeny in nursery and field, phenotyping, etc.
- A breeding program represent a certain **total cost** that depends on the cost of each basic operation and the number of each of these basic operations.
- The **dimensions of a program** refers to the number of these basic operations (number of crosses, size of the recruitment population).
- Relative efficiency is evaluated by comparing breeding programs representing the same total cost.
- Hence **dimensions have to be adjusted to have the same total** cost the programs. For example : if we do more genotyping in program B compared to program A, we have to save on phenotyping by decreasing the size of the recruitment population.

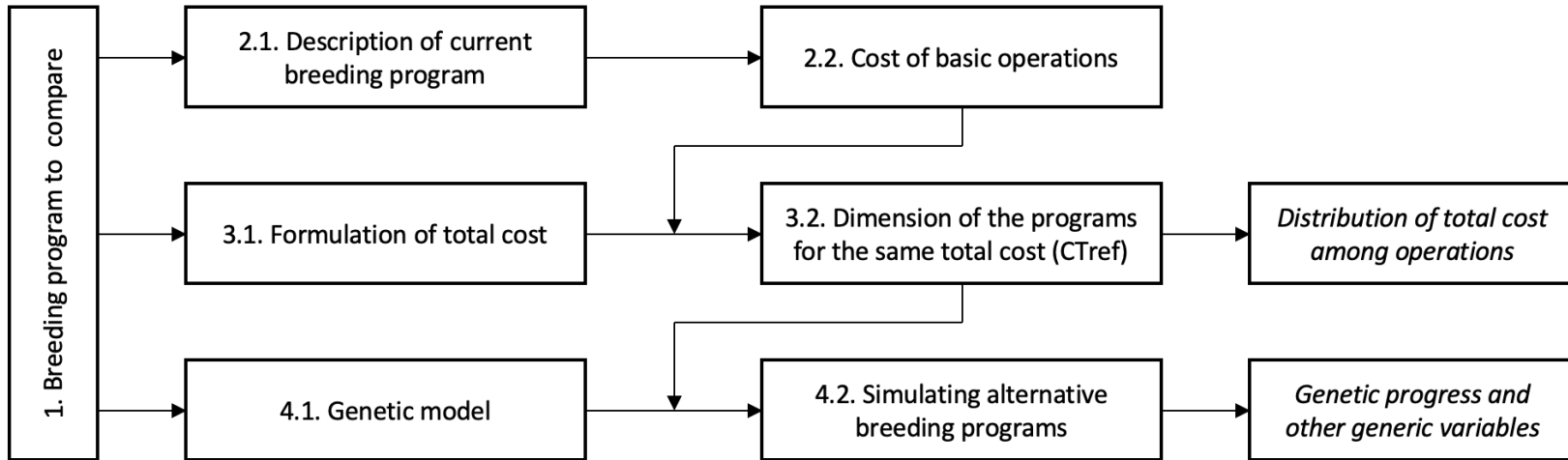


Multidisciplinary work with four main stages

1. What are the precise breeding programs that make sense to be compared, with the current state of knowledge, technology and specificity of current program.
2. Description of the basic operation that need to be made in the alternative breeding program. Evaluation of the cost of these operations.
3. Definition of the total cost as a function of the dimension of breeding programs and the cost of basic operations.
4. Comparison of breeding programs representing the same total cost, based on a predictive genetic model.



The detail sequence of work

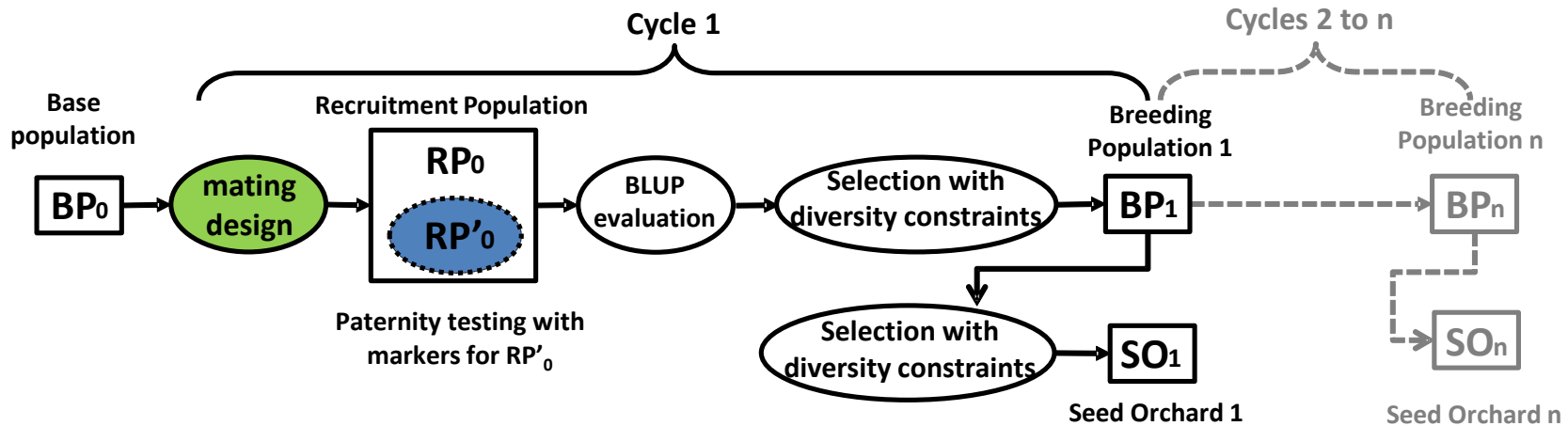


- Overlapping of stages 2, 3 and 4 over time.
- Lot of interdisciplinary learning during stages 1, 2 and 3 => wise to be modest on the breeding program to compare and modelled => reasonable on 4.1.



Application to Maritime Pine (work in progress) - Stage 1

- Comparison of 3 types of breeding programs which differ by the **mating design** and the **use of markers**:
 - CC: bi-parental crosses (no use of markers)
 - PMX: polymix crosses with paternity testing (use of markers)
 - OP: no crosses with paternity testing (use of markers)
- Same breeding cycle length (16 years) for the 3 breeding programs
- Similar to Bouffier *et al.* (2019) but the cost of each basic operation is taken into account (comparison for a given total cost)
- Genetic gains estimated with a genetic model = POPSIM (Mullin and Park, 1995)



Stage 2. Description of the current breeding program

- Work done with the engineers in charge of the breeding program (A. Raffin and F. Bernier)
- Description of the breeding scheme operations from initial crosses to the plantation of the selected parents in clonal archives
- Costs evaluation for each operation:
 - Staff expenditures: time spent and staff category (technician, researcher, engineer...)
 - Consumables costs and equipment rental
 - Services costs
 - Depreciation of equipment



Stage 2. Assumptions for cost evaluation

- No economies of scale
 - => The unit cost of one operation corresponds to the total cost for the corresponding operations divided by the the number of times this operation is carried out.
- We evaluate the research costs, independently from the origin of the funding of this research (INRAE, other partners...).
- The costs for trees evaluation don't take into account the costs of the operations realized by the forest owner.
- The main source of uncertainty in this cost evaluation is related to the staff cost. Hence, it is not necessary to be highly precise on other sources of cost, knowing this uncertainty.
- This cost evaluation can be useful for managers of the breeding programs. But **it's not an audit to optimize or reduce costs.**



Stage 2. Description of the operations

Year	Basic operation	Number	Time spent (in Man-Days)	Consumables	Rentals -Services	Depreciation
1	Crosses	50 crosses	40 MD (tech)	Pockets, protections, fuel, electricity	Boom lift	Cold room, oven
3	Seed sowing / Seedling growing	3000 genotypes	15 MD (tech) 0,5 man-day (engineer)	Peat clods, water, fertilizers, phytosanitary products		Shade house, irrigation system
4	Planting the seedlings in forest plots		45 MD (tech) 4 MD (engineer) Travel expenses	Stakes, labels, boundary markers		Thermal printer
12	Trees evaluation at 8 years		25 MD (tech) 1 man-day (engineer) Travel expenses		Pruning	Telescopic stick, input device.
16	Trees evaluation at 12 years		20 MD (tech) 0.5 man-day (engineer) Travel expenses			Measuring devices
16	Harvesting the selected trees	25 genotypes	15 MD (climbers) Travel expenses			Climbing equipment.
16	Grafting		19 MD (tech) 0.5 man-day (engineer)	Substrate, rootstock, labels, fertilizers, phytosanitary products		
16	Planting the selected clones in clonal archives		11.5 MD (techs) 0.5 MD (engineer) Travel expenses	Stakes, protection equipment, fuel, fertilizers		Scrub-clearing machine, spreader, plough, tractor
27	Pruning in clonal archives		1 MD (tech): trees labelling	Labels, springs	Pruning	
16-56	Clonal archives maintenance		0.5 MD (techs) per year		Pruning	Mulcher, tractor.
	Genotyping		Technicians and engineers	Test tubes, DNA extraction kits	Genotyping	



Stage 2. The variable cost of the operations

Basic operation	Year	Number	Total cost (€)	Unit cost (€)	% staff expenditures in total cost
Crosses	1	50	18 477	369.54	78 %
Seed sowing/seedling growing	3	3000	6 986	2.33	81 %
Planting the seedlings in forest plots	4	3000	19 797	6.60	91 %
Trees evaluation at 8 years	12	3000	12 984	4.33	73 %
Trees evaluation at 12 years	16	3000	9 041	3.01	82 %
Harvesting the selected trees	16	25	6 393	255.72	84 %
Grafting	16	25	7 621	304.84	93 %
Planting the clones in clonal archives	16	25	6 072	242.88	72 %
Pruning in clonal archives	27	25	860	34.40	42 %
Clonal archives maintenance	16-56	25	5 232	13.96	52 %
TOTAL VARIABLE COSTS			93 463		80 %
Genotyping				15.8	45 %



Stage 2. The additional fixed costs

- Fixed costs - independent from the size and the type of the breeding program:
 - Management of the breeding program: choice of the parents for the controlled crosses, installation of the recruitment population (forest plot), selection of the genotypes
 - Evaluation of the genetic values (BLUP estimation)

Basic operation	Time spent (in Man-Days)	Rentals -Services
Management of the breeding program	20 MD (engineer)	
Evaluation of the genetic values	10 MD (researcher) 10 MD (engineer)	TREEPLAN + statistical software license

- Total costs including variables and fixed costs

Basic operation	Total cost (€)	% staff expenditures
TOTAL Variable costs	93 463	80 %
TOTAL Fixed costs	46 017	70 %
TOTAL COST	139 480	77 %



Stage 3. Formulation of total cost

- Total cost of a breeding program with control crosses

$$CT^{CC} = N_C^{CC} \cdot C_C + N_R^{CC} \cdot C_R + N_P^{CC} \cdot (C_P + 1.1 \cdot C_G) + C_F$$

- Indexes : C = crosses ; R = recruitment population ; P = parents ; G = Genotyping
- C_k is the aggregated costs related to the the “aggregated” operation k

$$C_k = \sum_{i \in \Omega_k} \frac{c_i}{(1+r)^{t_i-1}}$$

- Ω_k : set of basic operation cost for the “aggregated” operation k
- r = discount rate. Capture the fact that one euro spent today is equivalent to $1/(1+r)$ euros spent the year after.



Stage 3. Discounted cost of basic operations

k	Corresponding basic operation (Ω_k)	t_i	$\frac{c_i}{(1+r)^{t_i-1}}$	C_k
Crosses (C)	Cross (controlled or polymix)	1	369.53	369.53
Candidate (R)	Seedling in nursery	3	2.15	12.50
	Plantation in forest	4	5.87	
	Phenotyping - 8 years old	12	2.81	
	Phenotyping - 12 years old	16	1.67	
Parent (P)	Harvest of selected candidates	16	141.99	531.96
	Grafting	16	169.26	
	Plantation in clonal archives	16	134.85	
	Pruning in clonal archives	27	12.40	
	Clonal archive maintenance	16-56*	73.45	
Genotyping (G)	Genotyping	16	8.77	8.77



Stage 3. Cost of basic operations with various discount rate

Cost	r=0%	r=4%	r=10%
C_C	369.53	369.53	369.53
C_R	12.50	16.27	9.12
C_P	531.96	1047.07	214.86
C_G	8.77	15.80	3.78

- The discount rate does not affect the cost related to the crosses because these crosses occur the first year.
- The weight of the other operations that occur later (R, P and G) decreases as the discount rate increases. Ex : C_R/C_C ; C_P/C_R and C_P/C_C decreases.



Stage 3. Total cost for each breeding program

- Control crosses : 110% of the parents are genotyped to guarantee the pedigree

$$CT^{CC} = N_C^{CC} \cdot C_C + N_R^{CC} \cdot C_R + N_P^{CC} \cdot (C_P + 1.1 \cdot C_G) + C_F$$

- Polymix : only a portion of the recruitment population is genotyped. This proportion represent 5 times the number of parents.

$$CT^{PMX} = N_C^{PMX} \cdot C_C + N_R^{PMX} \cdot C_R + N_P^{PMX} \cdot (C_P + 5 \cdot C_G) + C_F$$

- Open polynisation : no cost related to crosses. Only a portion of the recruitment population is genotyped.

$$CT^{OP} = N_R^{OP} \cdot C_R + N_P^{OP} \cdot (C_P + 5 \cdot C_G) + C_F$$



Stage 3. Defining the dimensions of alternative breeding program

- Dimensions (N_P , N_G , N_C , N_R) of each breeding program (CC, PMX, OP) adjusted to fit the same total cost (CT^{REF})
- Example with CC breeding programs:

$$CT^{CC} = CT^{REF} \Leftrightarrow N_R^{CC} = \frac{CT^{REF} - C_F}{C_R} - N_C^{CC} \cdot \frac{C_C}{C_R} - N_P \cdot \frac{C_P + 1.1 \cdot C_G}{C_R}$$

- Number of parents: $N_P = 150$ for all breeding programs (simplification to fulfil genetic diversity constraints related to the breeding population and the seed orchards)
- Number of trees genotyped: $N_G = 1.1 \times 150 = 165$ (to check id considering 10% of id errors)
- N_C and N_R : several levels of N_C were considered (from 50 to 300) and N_R was adjusted in consequence
 - If $N_C = 50 \rightarrow N_R = 17\,956$
 - If $N_C = 300 \rightarrow N_R = 10\,566$



Stage 3. Dimension of alternative breeding program

Breeding Program	Name	Size variables			
		N_C	N_R	N_P	N_G
CC	CC50	50	17956	150	165
	CC100	100	16478	150	165
	CC150	150	15000	150	165
	CC300	300	10566	150	165
PMX	PMX50	50	17546	150	750
	PMX100	100	16068	150	750
	PMX150	150	14590	150	750
OP	OP150	0 (seeds collected on 150 mothers)	19024	150	750
	OP50	0 (seeds collected on 50 mothers)	18498	150	1500

N_C = nber of crosses
 N_R = size of the recruitment population
 N_P = nber of parents
 N_G = nber of trees genotyped

Breeding programs are generally compared at a given recruitment population size. In this study, comparison carried out at a given total cost.



Stage 3. Distribution of total cost among operations

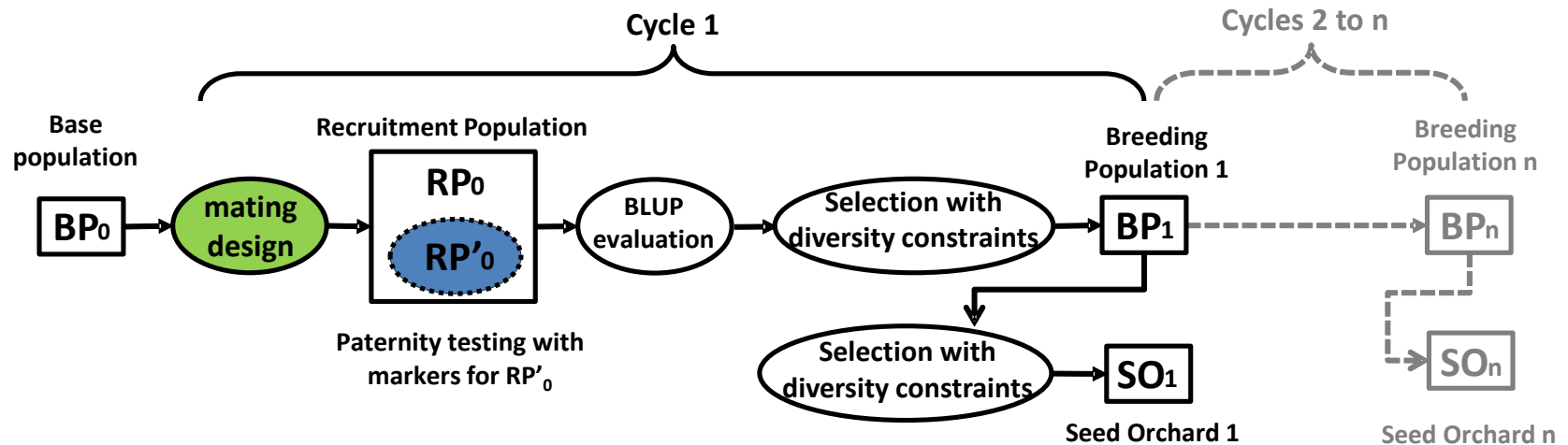
Breeding Program	Name	Distribution of total cost			
		Crosses	Candidates	Parents	Genotyping
CC	CC50	5.7%	69.2%	24.6%	0.4%
	CC100	11.4%	63.5%	24.6%	0.4%
	CC150	17.1%	57.8%	24.6%	0.4%
	CC300	34.2%	40.7%	24.6%	0.4%
PMX	PMX50	5.7%	67.7%	24.6%	2.0%
	PMX100	11.4%	62.0%	24.6%	2.0%
	PMX150	17.1%	56.3%	24.6%	2.0%
OP	OP150	0.0%	73.4%	24.6%	2.0%
	OP50	0.0%	71.3%	24.6%	4.1%

- Phenotyping (candidates) is the main cost: from 40% to 73% of total cost
- Genotyping cost is marginal
- Trade-off between nber of crosses and size of the recruitment population



Stage 4. Genetic modeling

- POPSIM software designed specifically for simulations of tree breeding programs over multiple generations
- Based on stochastic samplings through a parametric genetic model



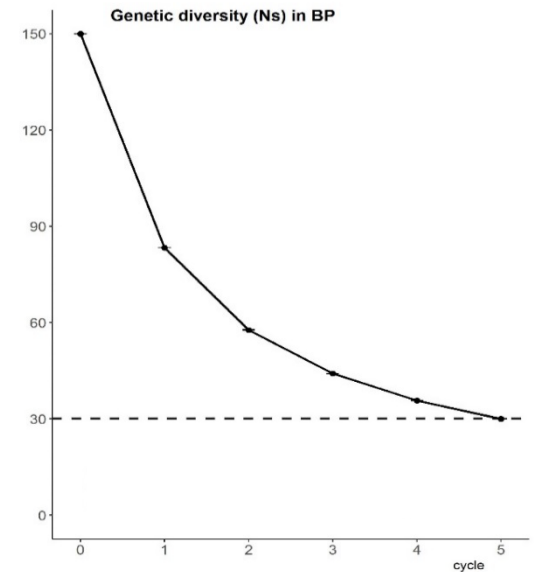
Stage 4. Genetic modeling

- Main advantages:
 - freely available on Skogforsk website
 - can handle large range of tree mating designs (some with paternity recovery)
 - BLUP estimation with ASReml
 - maximize genetic gain at a predetermined level of genetic diversity (OPSEL)
- Limitations:
 - BP size identical over generations
 - discrete generations (no overlapping)
 - PMX limits (polymix defined is mated with the same set of mothers)



Stage 4. Genetic modeling (parameters)

- Single trait: $CVa=15\%$ and $h^2=0.2$
- Simulations carried out over 5 breeding cycles (with 10 to 25 iterations)
- Diversity constraints: $N_s \geq 30$ in the breeding population after 5 cycles and $N_s=10$ in seed orchards whatever the generation
- Breeding program comparison based on genetic gain in seed orchard after 5 cycles



Stage 4. Comparison of genetic gain among breeding programs

		# crosses	# offspring per family	Genetic gains at cycle 5 (sd)	# ite	simulation time
CC	CC50	50	359	_*	-	-
	CC100	100	165	78.8% (2.5%)	10	5h41
	CC150	150	100	79.4% (3.2%)	25	14h20
	CC300	300	35	76.7% (3.8%)	25	24h16
PMX	PMX50	50	349	70.8% (4.4%)	25	14h28
	PMX100	100	160	76.3% (3.6%)	25	14h30
	PMX150	150	97	78.8% (3.8%)	10	5h50
OP	OP150	150 mothers	127	78.9% (4.7%)	25	10h18
	OP50	50 mothers	370	72.6% (4.7%)	10	5h52

* diversity constraints (Ns) can not be fulfilled



Stage 4. Main lessons from the comparison of breeding scheme

- Similar level of genetic gains whatever the breeding program (CC, PMX, OP)
 - Higher number of FS families (PMX, OP) do not increase genetic gains
 - Trade-off number of families vs. size of each family (and trade-off between vs. within family selection)
- Diversity is easier to manage with PMX/OP breeding program
 - Number of crosses in CC breeding programs must be sufficient to fulfil diversity constraints (which is not the case for PMX and OP)
 - When 20% of crosses are unsuccessful (additionnal simulations not shown), CC150 can not fulfil diversity constraint whereas OP150 can (genetic gain for OP150 only decrease slightly from 78.8% to 75.7%)
- Genotyping costs is a limited extra-cost
 - Open opportunities for new breeding strategies based on high number of trees genotyped



Perspectives on Maritime Pine (additional simulations)

- **Number of parents** (25% of total cost)

The same diversity constraints could be achieved with a different number of parents

→ simulation of breeding programs with other values for N_p

- **Diversity constraints** ($N_s \geq 30$ in the breeding population and $N_s = 10$ in seed orchard)

Sensitivity of diversity constraints on the breeding programs ranking? → simulation of the same breeding programs with other diversity constraints

- **Total cost** (CT^{REF})

Higher / lower total cost could affect differentially the breeding programs

