Optimum Hybrid Maize Breeding Strategies Using Doubled Haploids

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Outline

- **Introduction**
- ***In vivo* induction of maternal haploids**
- **DH-line based breeding schemes**
- **Software MBP for optimizing the allocation of breeding resources**
  - **Features**
  - **Selected results**
- **Summary and conclusions**

Applications of the DH technology

- **Marker-trait association studies**
- **Marker-aided introgression of genes**
- **Genetic engineering**
- **Molecular cytogenetics**
- **Hybrid breeding**

➢ **The DH technology has become an indispensable tool of modern maize research and breeding**

Advantages of DH lines in hybrid breeding

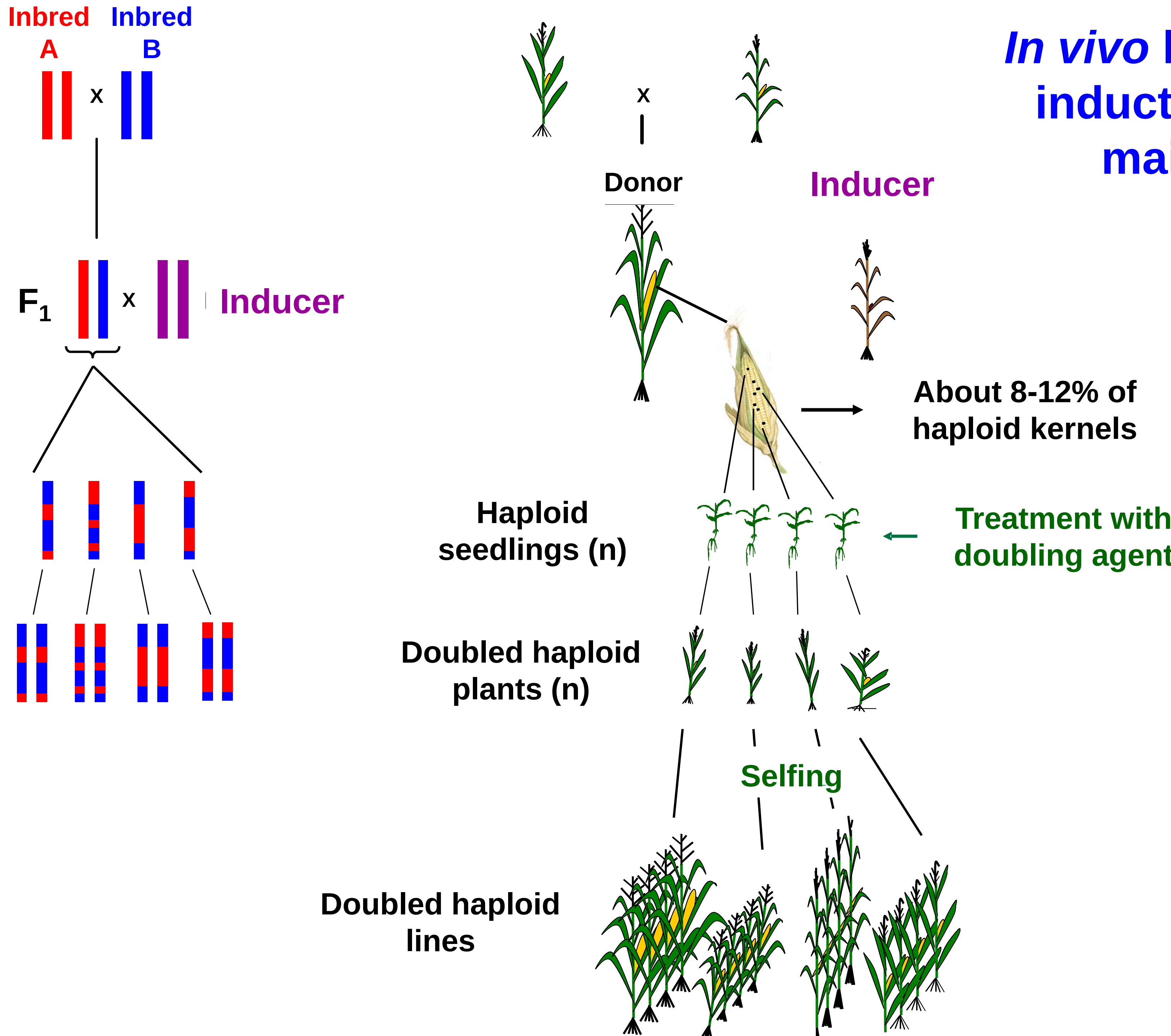
- **Maximum genotypic variance in line-*per-se* and testcross trials**
- **High reproducibility of early-testing results**
  - **Increased selection gain**
- **Complete homozygosity from the very first generation**
  - **Perfect compliance with DUS criteria for variety protection, short "time to market"**
  - **Reduced nursery expenses, simplified logistics**
  - **Facilitates marker-assisted selection and backcrossing**

In vivo induction of maternal maize haploids

For review see **Geiger (2009) *in* Handbook of Maize. Springer, New York**

- **Pollination of maize plants with specific genotypes called inducers, which leads to kernels with a haploid embryo and a regular triploid endosperm**
- **Widely used for line development in commercial hybrid maize breeding**
- **Increasingly used in research**
- **Only moderate influence of donor genotype and induction environment compared to *in vitro* haploid induction**

In vivo haploid induction in maize



Haploid-identification markers (1)

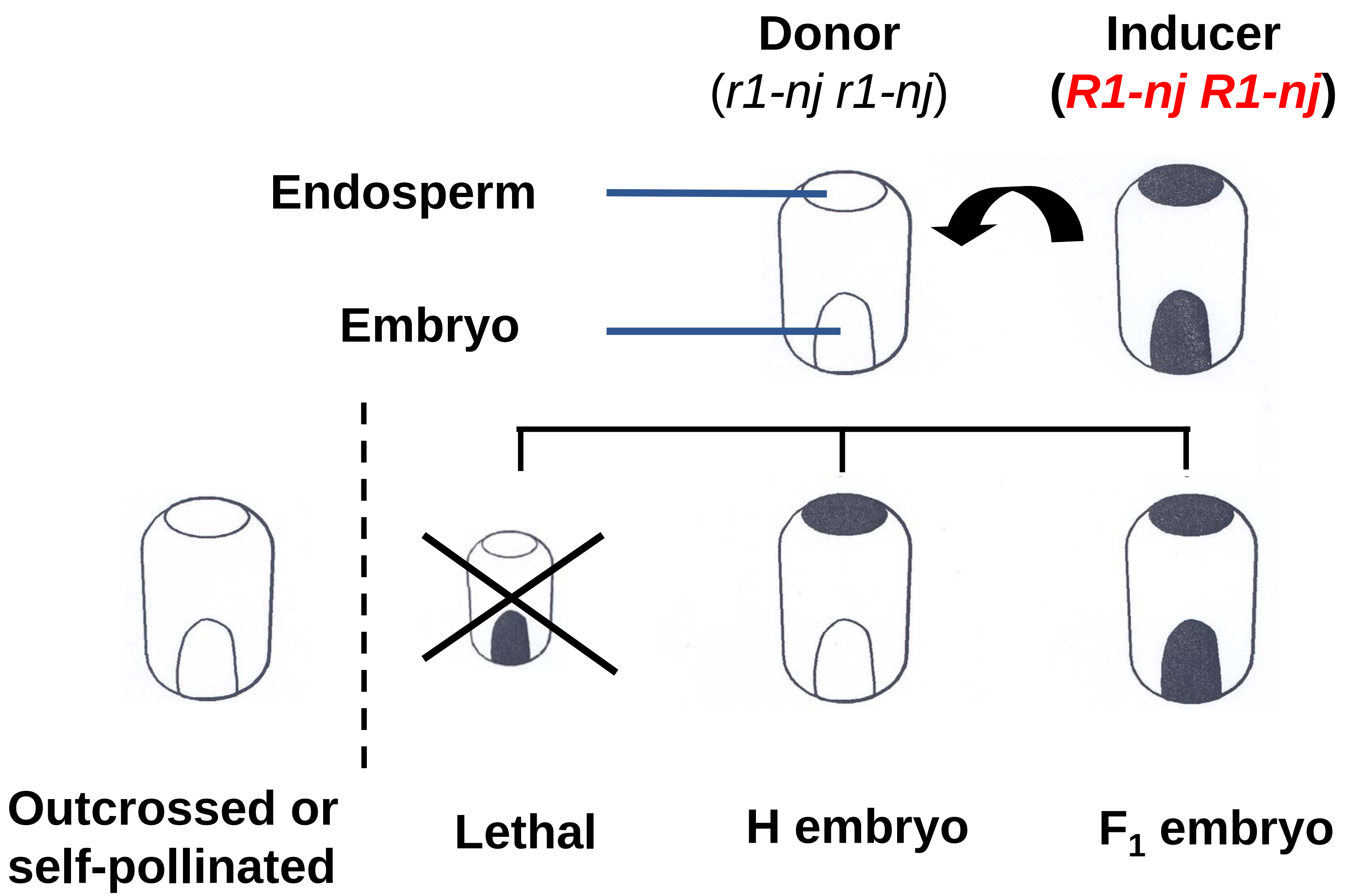
Markers expressed before chromosome doubling

- **Dominant grain color marker gene *R1-nj* (in conjunction with mutant pigmentation genes *A1* or *A2,* and *C2*)**
  - **Causes pigmentation in the aleurone (endosperm) and in the scutellum (embryo tissue)**
  - **Needs donor with colorless seeds**
  - **Expression may be suppressed by inhibitor genes (e.g. *C1-I*) carried by the female parent**
- **Dominant color marker genes expressed in the primary root and coleoptile (e.g. *Pl1* in conjunction with *B1*)**

Haploid-identification markers (2)

„Red crown“ marker *R1-nj*

(After Röber 1999)



„Red crown“ marker *R1-nj (3)*

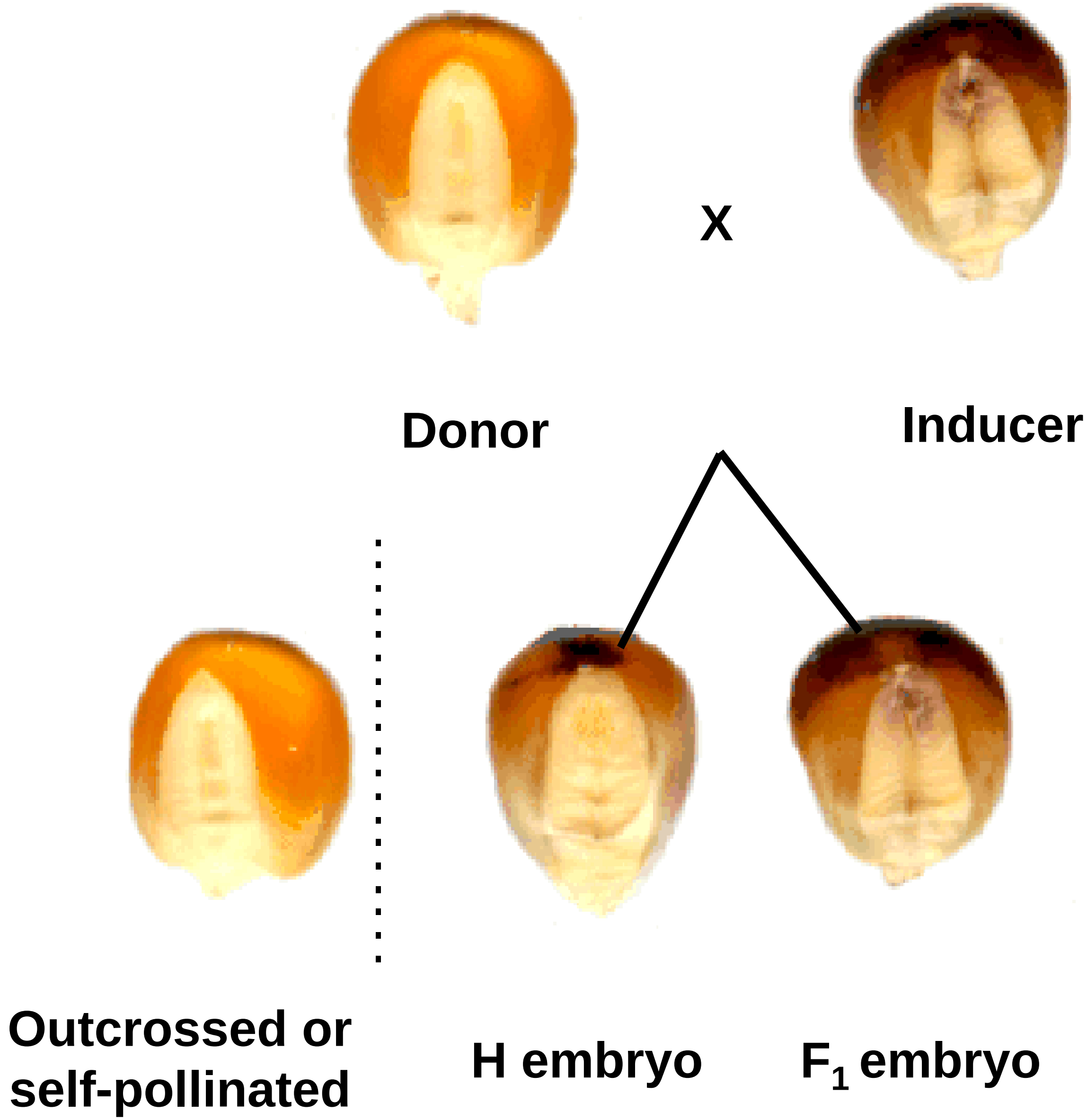


Photo F.K. Röber

Chromosome doubling

- **Immersion of seedlings in colchicine (Gayen *et al.* 1994, Deimling *et al.* 1997, Eder and Chalyk 2002)**
  - **70 – 80% of the seedlings survive**
  - **10 – 40% of the surviving plants produce selfed seed**

- **Due to high toxicity of colchicine, most breeding companies are interested in less hazardous substances**
  - **Herbicides, e.g. Pronamid, APM, Trifluralin, Oryzalin**
  - **Nitrous oxide gas (Kato 2002)**

  -> not suited for large-scale haploid induction

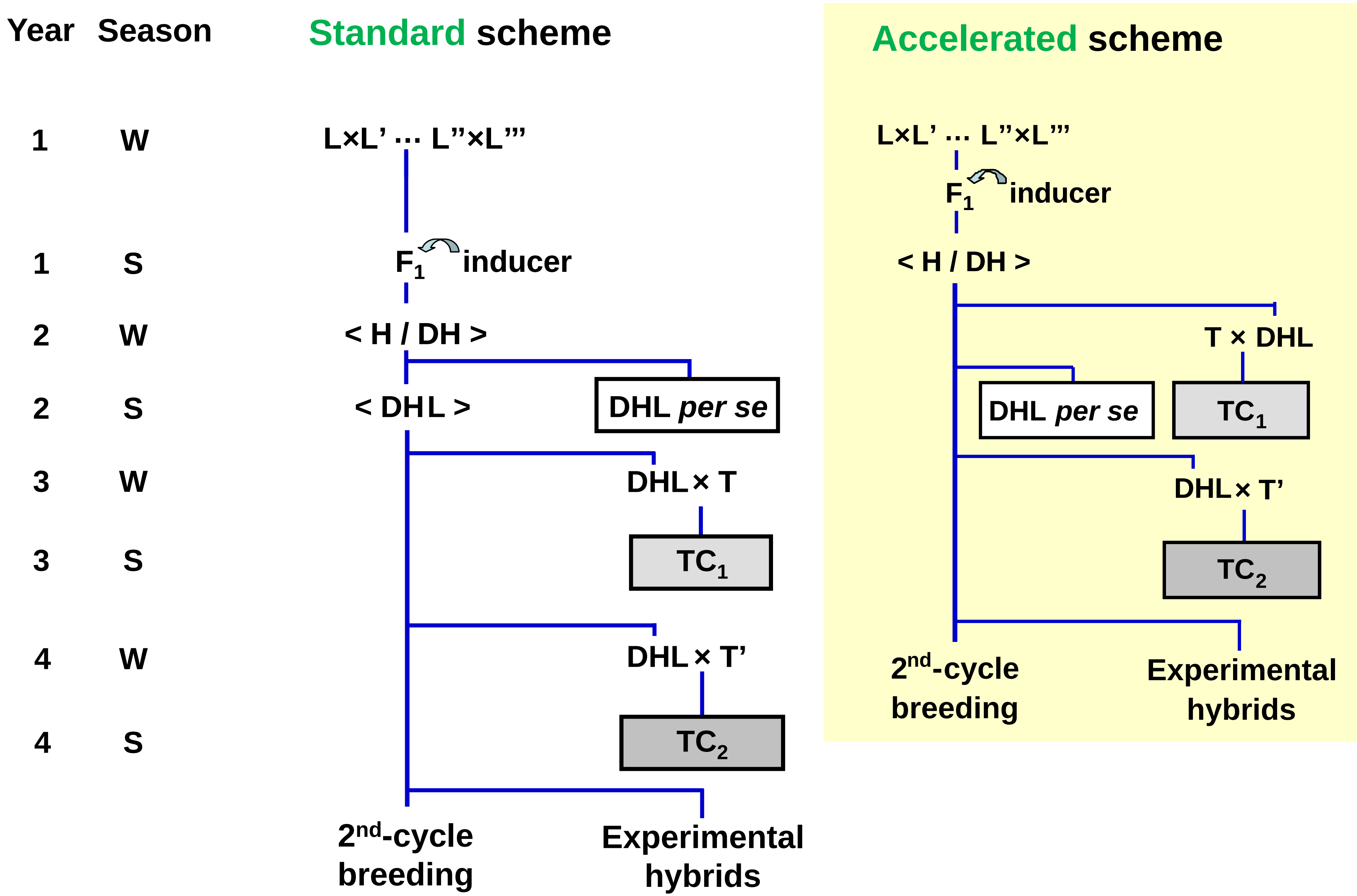
Seedling ready for reducing the root and clipping the tip of the coleoptile

Transplanting colchicinised juvenile plants into the field

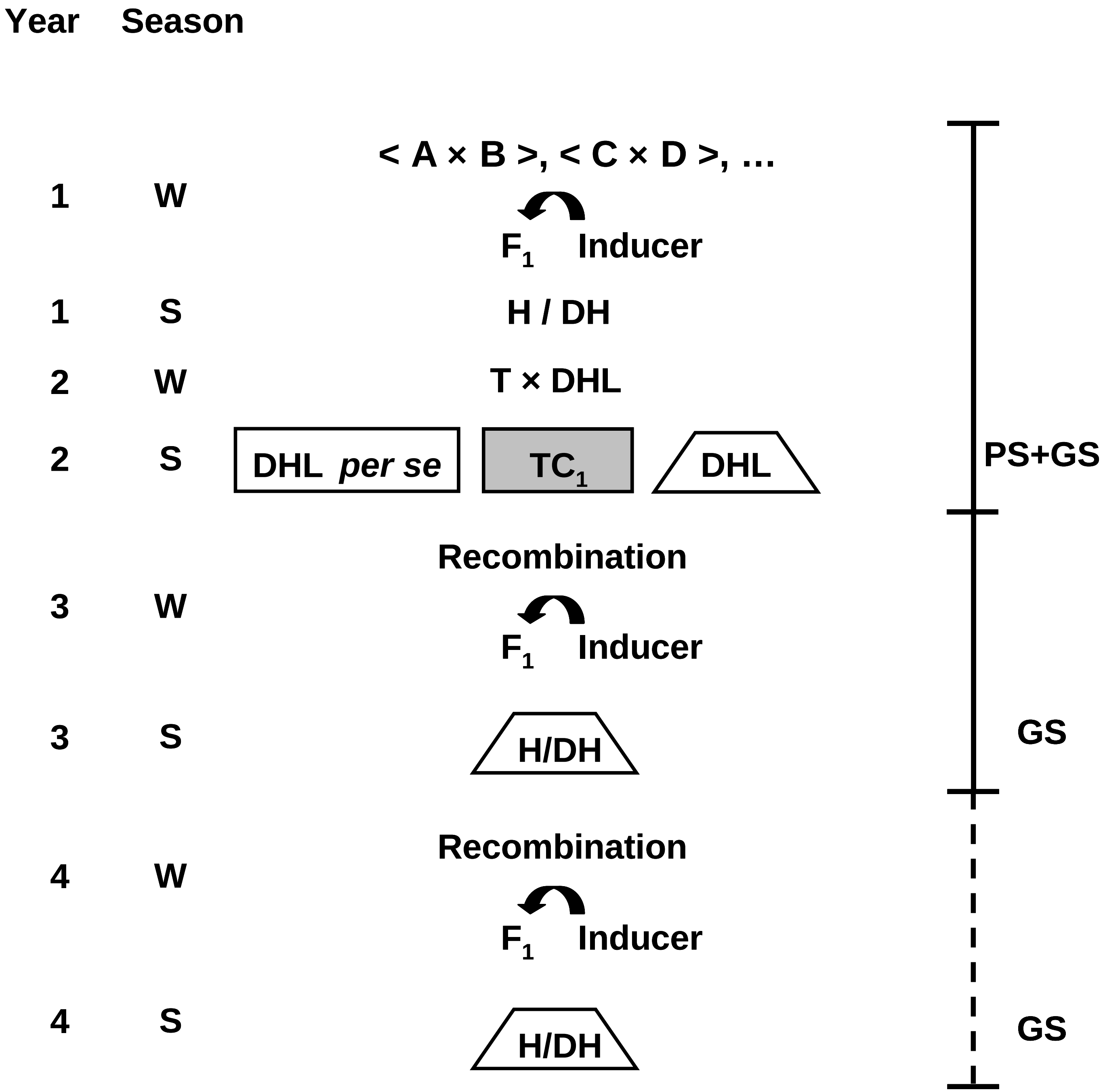
DH-line observation nursery

Photo W. Schmidt

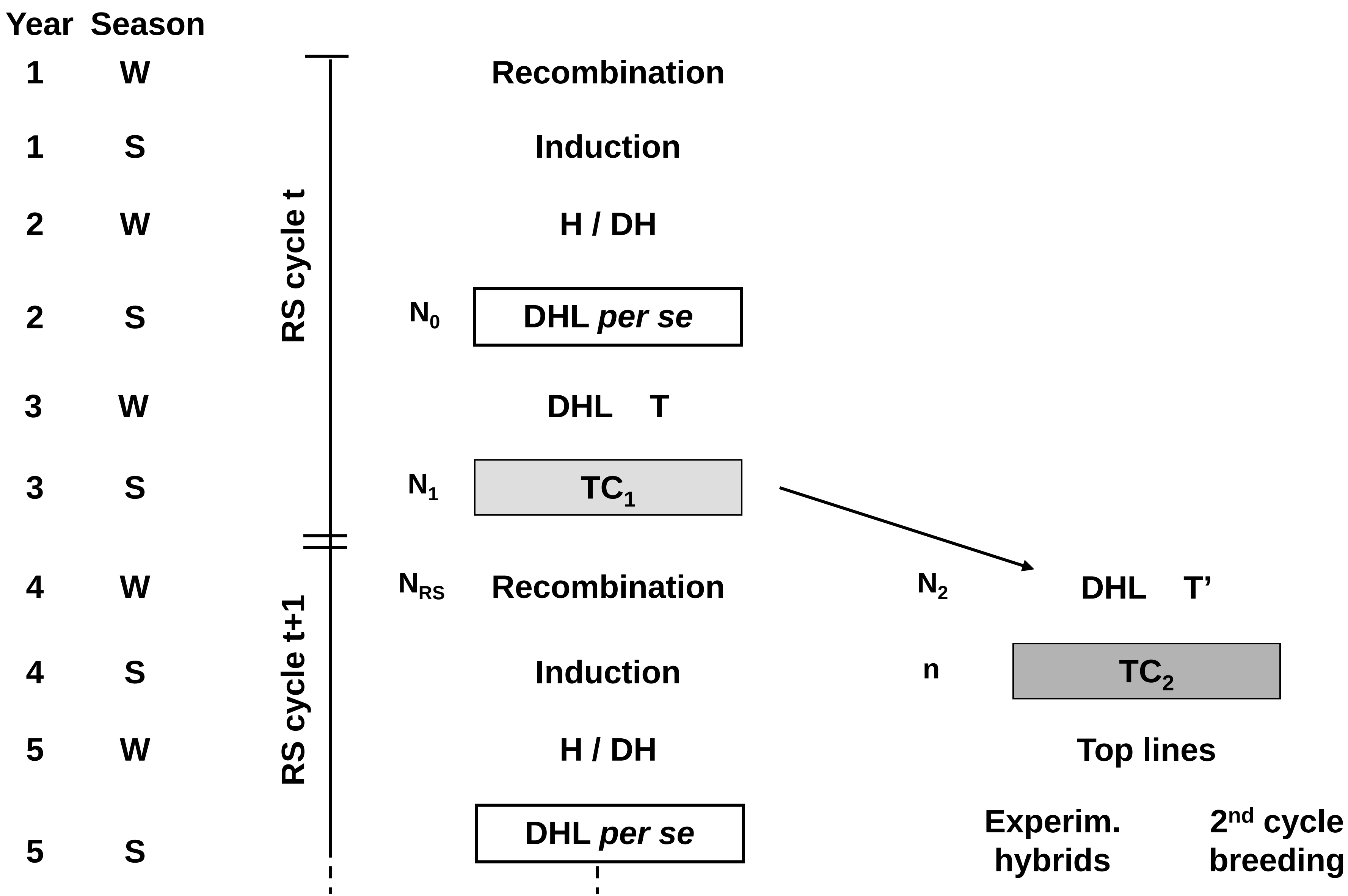
Two-stage line development (LD) schemes



Integration of genome-wide selection (GS)



Integrated recurrent selection (RS) and parent line development (LD)



MBP (Version 1.0)

Software for optimizing Maize Breeding Plans based on DH lines

(Gordillo & Geiger 2008)

- **Maximizes the expected genetic gain per year for a given annual budget and a limited relative annual loss of genetic variance.**
- **Allows to optimize 1-, 2-, and 3-stage testcross selection procedures for alternative breeding schemes.**
- **Allows the user to specify the tester type (*e.g.* pure line, single cross, population) for each testcross selection stage separately.**
- **Accounts for detailed monetary costs of each individual breeding step.**

MBP is applicable to :

- **Line development (LD) in hybrid breeding**
- **Recurrent selection (RS)**
- **Integrated RS/LD approaches**

  - **RS is treated as an integral part of LD**

- **Interlinking successive staggered breeding cycles**

MBP: Quantitative genetic and operational input variables

- **Estimates of variance components and genetic correlation coefficients**
- **Haploid induction parameters**
- **Costs of the individual breeding steps**

➢ **All variables are based on data from collaborating breeding companies and can be varied by the user according to his genetic, technical, and financial resources.**

MBP: Gain criterion

The gain criterion is the expected genetic gain in GCA for an index composed of the testcross performance for grain yield and dry matter content.

- **Arbitrary index weights may be chosen by the user.**

Selected MBP results: 1. General program specifications

- **Annual budget: US $ 750,000**
- **Proportion of lines pre-selected for *per se* performance: 50%**
- **Single-cross tester(s) at all selection stages**
- **Yield trials: multiple locations, unreplicated**
- **Three finally selected lines per LD cycle (N_{LD} = 3)**
- **Annual loss of genetic diversity restricted to 2%**
- **Gain criterion:**

  I = Grain yield (Qx/Ha) + 2.5 Dry matter content (%)

MBP results: 2. Standard *vs*. accelerated two-stage LD scheme

Scheme		Optimum allocation[a]						Genetic gain for yield (kg ha^{-1})	
	N_{LD}	N_1	N_2	T_1	T_2	L_1	L_2	per cycle	per year
Standard (4 ys)	3	3573	57	1	7	5	20	812	203
Acceler. (3 ys)	3	5680	79	1	6	3	19	786	262

[a] N, T, L = Number of DH-lines, testers, and locations, respectively
Indices refer to test stages 1 and 2

Note: In the Accelerated Scheme, the *per se* evaluation of DH lines is not before but in parallel to the TC_1

→ 7146 vs. 5680 lines are evaluated *per se* in the standard vs. accelerated scheme, respectively

- High-input, short-cycle breeding procedures maximize the genetic gain per year.

MBP results: 3. Standard *vs*. accelerated 1- and 2-stage RS

Annual loss of genetic variance restricted to 2%

Scheme		Optimum allocation						Genetic gain for yield (kg ha^{-1})	
	N_{RS}	N_1	N_2	T_1	T_2	L_1	L_2	per cycle	per year
1-stage RS:									
Stand. (3 years)	43	2481	-	2	-	6	-	504	168
Acceler. (2 years)	57	3900	-	1	-	8	-	442	210
2-stage RS:									
Stand. (4 years)	32	3821	225	1	3	4	12	624	156
Acceler. (3 years)	39	5574	274	1	3	3	12	576	192

- The accelerated version is more efficient than the standard scheme.
- One-stage RS is superior to two-stage RS.
- In the most efficient RS scheme more than 50 DH lines need to be recombined to comply with the loss-of-genetic-variance restriction.

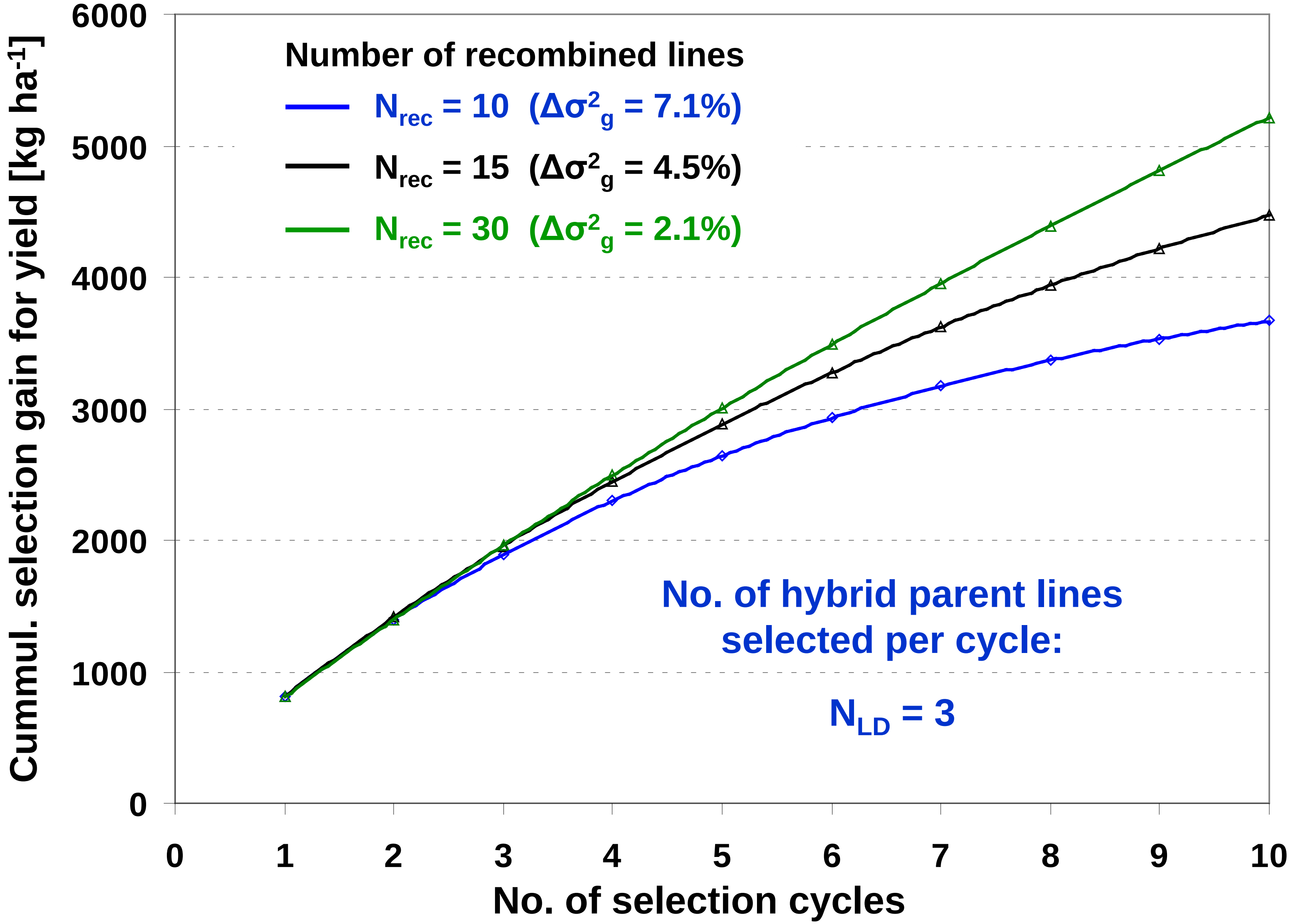
MBP results: 4. Integrated RS/LD standard breeding scheme; influence of the weights for RS and LD

- One-stage testcrossing in RS
- Two-stage testcrossing in LD
- Annual loss of genetic variance restricted to 2%

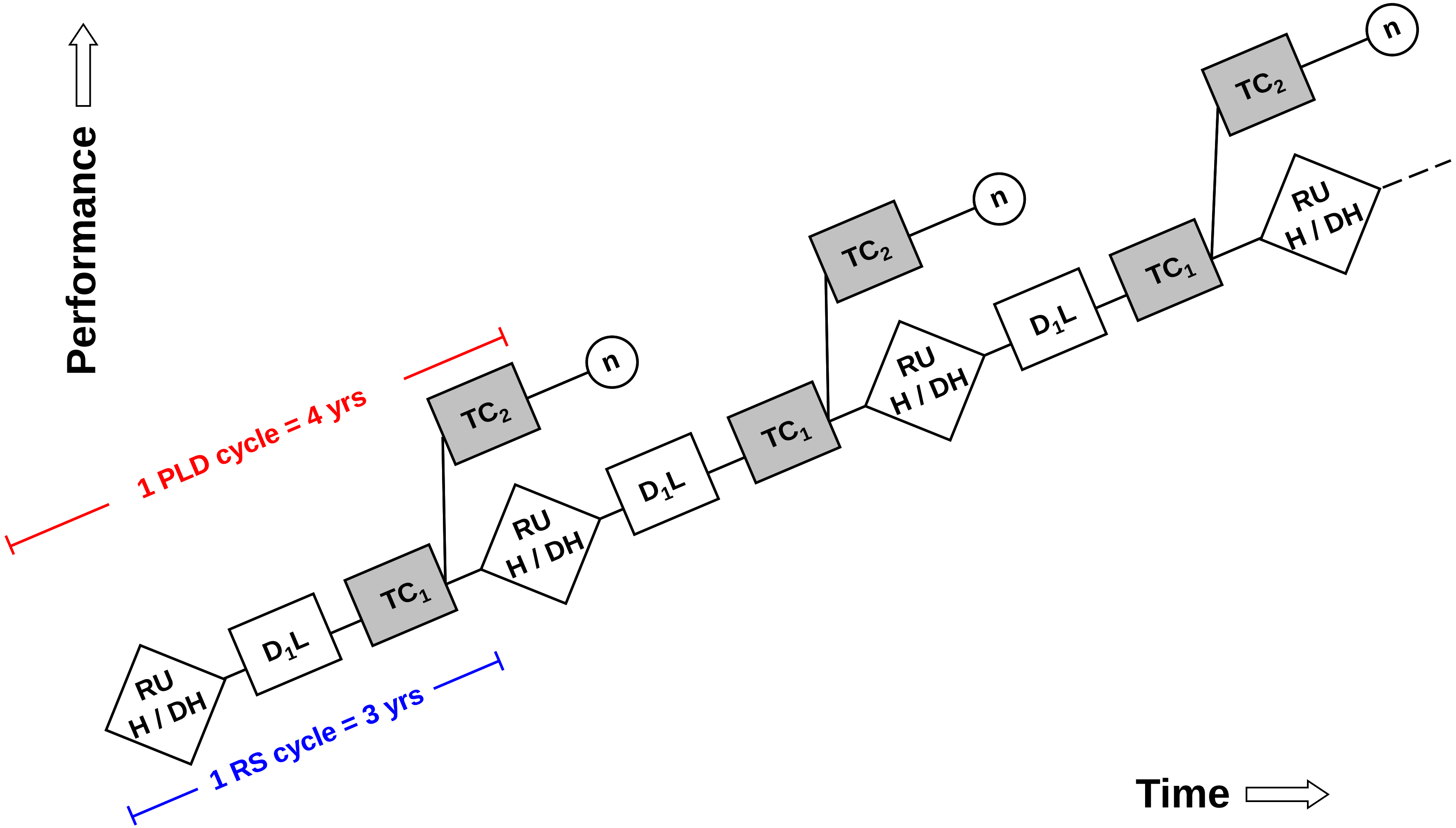
			Optimum allocation						Genetic gain for yield (kg ha^{-1}) per year	
w_{RS} : w_{LD}	N_{RS}	N_{LD}	N_1	N_2	T_1	T_2	L_1	L_2	G_{RS}	P_{LD}
0 : 1	34	3	3573	57	1	7	5	20	143	204
0.5 : 0.5	32	3	3805	153	1	4	4	14	155	202
1 : 0	32	3	3831	225	1	3	4	12	156	199

➢ Weights given to RS and LD, respectively, considerably influence the optimum allocation but hardly affect the maximal genetic gain per year.

MBP results: 5. Influence of population size on the long-term progress in line development



Relative importance of recurrent selection and parent line development



Summary and Conclusions (1)

- ***In vivo* techniques of haploid induction have become standard tools in maize breeding and research.**

- **Major advantages of DH lines in hybrid breeding include**
  - **maximum genetic variance from the very first generation**
  - **perfect compliance with DUS criteria**
  - **short time to market**
  - **simplified logistics**
  - **reduced expenses for selfing and maintenance breeding.**

Summary and Conclusions (2)

- **Genome-wide selection can effectively be integrated in DH-line based breeding schemes.**
- **A software package “MBP (version.1.0)” has been developed to optimize the allocation of breeding resources and to determine the relative merits of alternative breeding schemes.**
- **High-input, short-cycle, breeding schemes are expected to provide maximal annual genetic gain.**

Summary and Conclusions (3)

- **The long-term genetic gain in LD builds up on the cumulative genetic gain from RS.**
  - **It is advisable to weight RS higher than LD when optimizing *combined* RS/LD breeding schemes**
- **Increasing the weight for RS leads to a considerable increase in the gain from RS, while it hardly affects the gain in LD.**
- **Sizable numbers of selected DH-lines need to be recombined to preserve enough genetic diversity for subsequent breeding cycles, especially in case of short-cycle (accelerated) schemes!**

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