



# From wild relative to relative - making pre-breeding matter

## **Regional training workshop**


*Predictive characterization and pre-breeding of crop wild relatives*

Pretoria, South Africa

Monday 13<sup>th</sup> – Thursday 16<sup>th</sup> April 2015

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South Africa**



**“Food is the moral right of  
all who are born into  
this world.”**

**– Norman Borlaug,**  
(March 25, 1914-Sept. 12, 2009),  
Nobel laureate and father  
of the Green Revolution



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# SOUTH AFRICAN SEED MARKET FOR AGRONOMIC CROPS 2012/13

CROP	Mass (kilogram)		MARKET VALUE BASED ON RETAIL SELLING PRICE [seed price list] R million	Mass (kilogram)		
	Local market	Export market		GMO's (local market)	Hybrids (local and inter- national markets)	Open-pollinated [non-hybrids] (local and international markets)
Barley	6 710 138	0	28.93	0	0	6 710 138
Canola	322 291	400	34.30	0	43 610	279 081
Cotton	44 275	382 500	3.94	405 436	0	426 775
Dry bean	2 870 192	634 107	93.88	0	0	3 504 299
Dry pea	300	128 675	1.04	0	0	128 975
Durum Wheat	172 975	0	0.04	0	0	172 975
Grain sorghum	529 777	267 958	25.42	0	517 045	280 690
Groundnut	2 657 682	43 500	48.06	0	0	2 701 182
Kidney bean	650	0	0.05	0	0	650
OP Maize (white)	2 517 151	1 293 641	46.12	0	0	3 810 792
OP Maize (yellow)	1 091 876	12 115	43.90	0	0	1 103 991
Soya bean	5 789 324	226 300	127.49	2 835 275	0	6 015 624
Sugar beet	0	0	0.00	0	0	0
Sunflower	2 151 705	611 599	209.95	0	1 741 823	1 021 481
Tobacco	12	141	2.80	0	101	52
Wheat	14 837 145	184 325	115.39	0	0	15 021 470
Other Agronomy Crops			3.42			
HYBRID MAIZE: 60K UNITS	60K UNITS	60K UNITS		60K UNITS	60K UNITS	
Yellow hybrid: Commercial market	955 147	20 034	1 721.28	727 924	247 257	0
Yellow hybrid: Smallholder market	1 789	16 648	6.71	0	18 437	0
White hybrid: Commercial market	715 179	55 648	1 755.90	606 005	164 822	0
White hybrid: Smallholder market	5 445	76 774	27.51	320	81 899	0
<b>TOTAL</b>			<b>R 4 296.12</b>			

# Overview

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- **Introduction**
  - **Wheat**
  - **Wheat rusts**
  - **Resistance**
  - **Conventional breeding**
- Wheat pre-breeding
- Case study
- Current and future work



# Wheat

## Introduction/



- Global wheat production needs to increase
- Stabilize/Increase crop productivity under:
  - Increasingly unstable climate;
  - Less hectares; and
  - Under increase biotic and abiotic stresses
- Breeding and agronomic practices important
- Abiotic: drought, salinity and heat
- Biotic: Fungal diseases most important biotic stressors

# Wheat rusts

## Introduction/



- Wheat rust pathogens
  - *Puccinia graminis* f. sp. *tritici*
  - *Puccinia triticina*
  - *Puccinia striiformis* f. sp. *tritici*
- Causes big crop losses in some seasons
- Breeding for resistance will help in sustaining wheat farming
- Less fungicide application
  - Lowers production costs
  - Decreases environmental impact of production



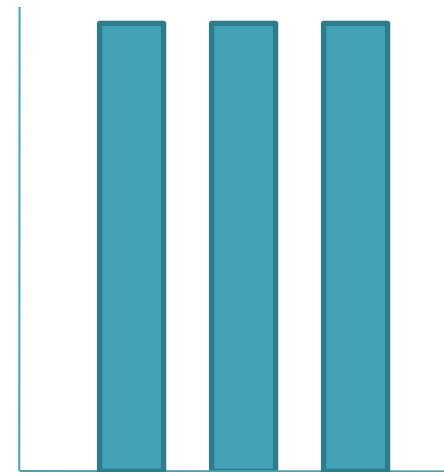


# Resistance breeding

## Introduction/



- Vertical resistance
  - Race specific resistance, major gene resistance
  - Gene-for-gene
  - Pathotypes evolve quicker to overcome the resistance



Race- specific resistance



# Resistance breeding

## Introduction/



- Vertical resistance
  - Race specific resistance, major gene resistance
  - Gene-for-gene
  - Pathotypes evolve quicker to overcome the resistance
- Horizontal resistance
  - Race non specific , minor gene resistance or durable resistance
  - Minor genes with accumulating effect
  - Resistance more complex for pathotypes to overcome



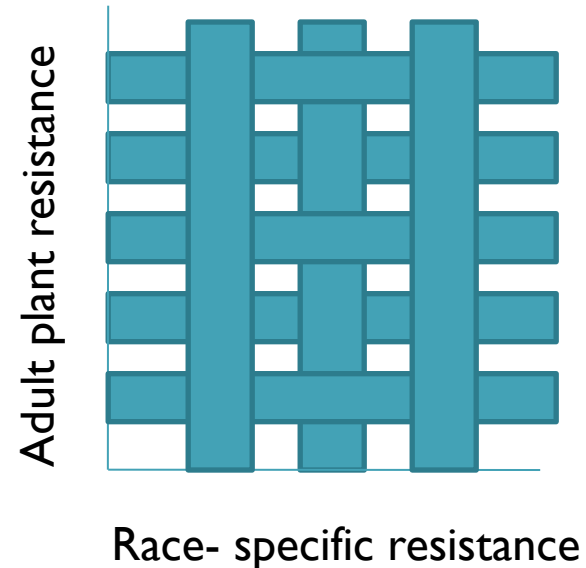
Adult plant resistance

# Resistance breeding

## Introduction/



- Vertical resistance
  - Race specific resistance, major gene resistance
  - Gene-for-gene
  - Pathotypes evolve quicker to overcome the resistance
- Horizontal resistance
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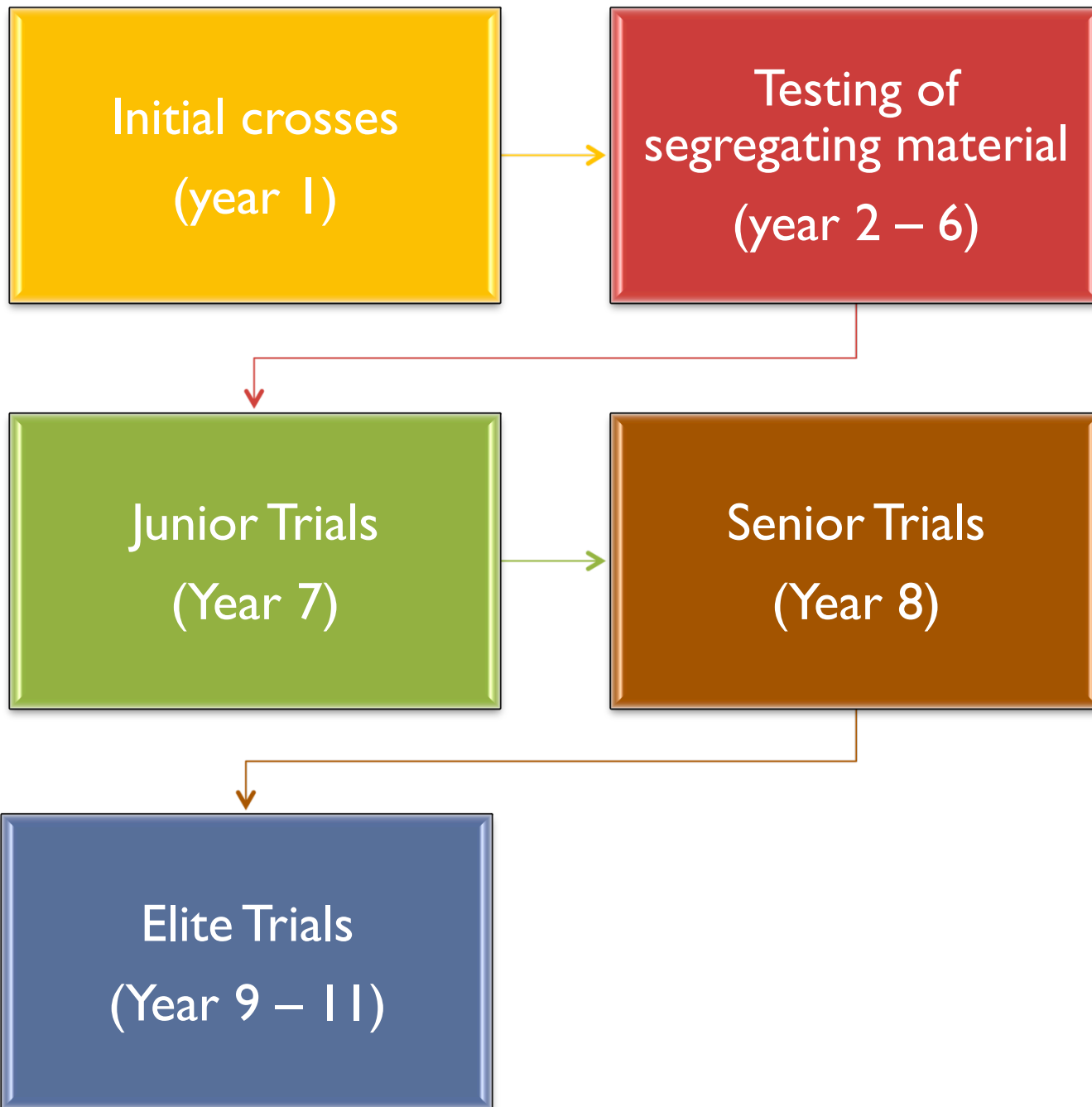


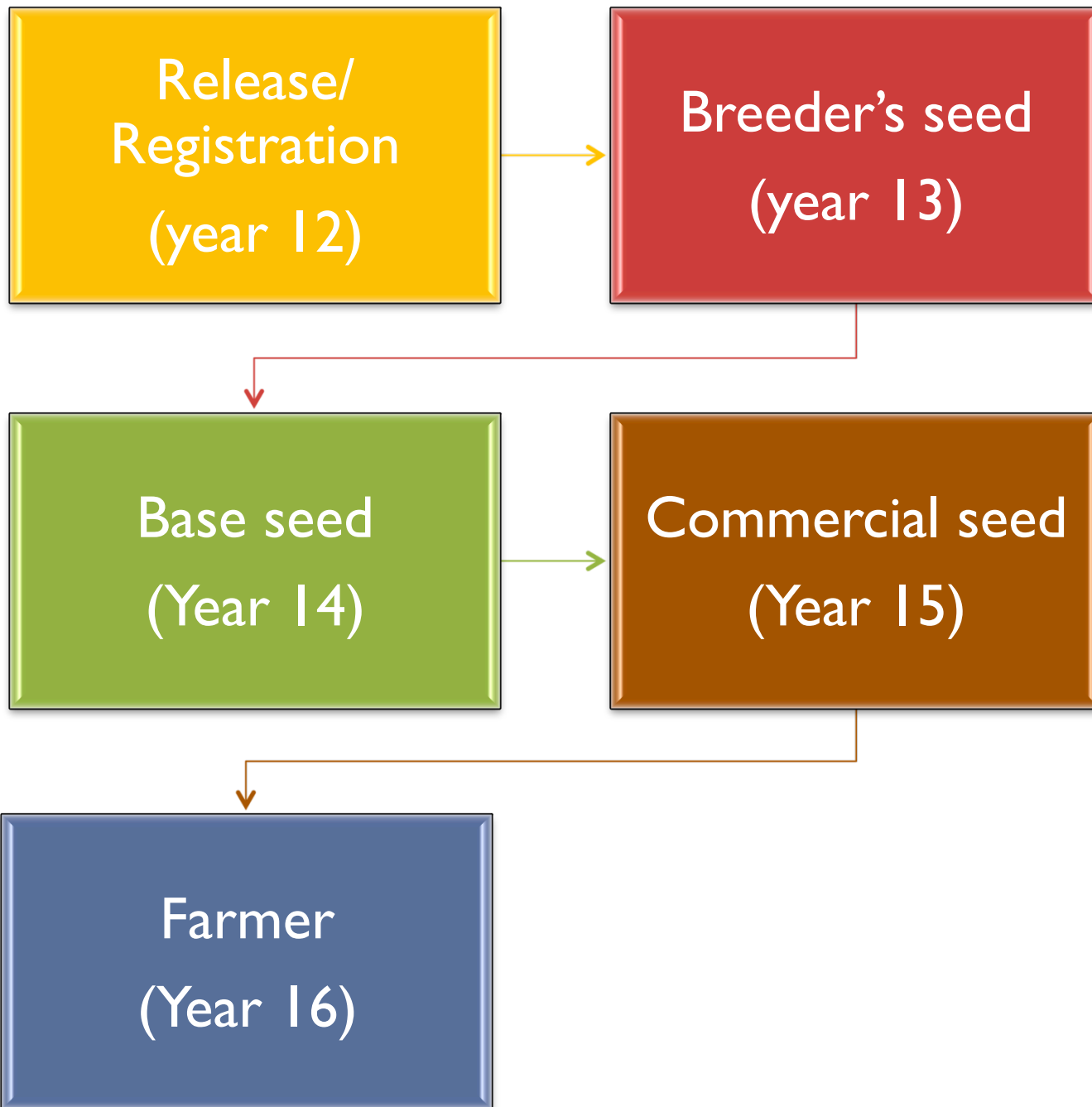
# Resistance breeding

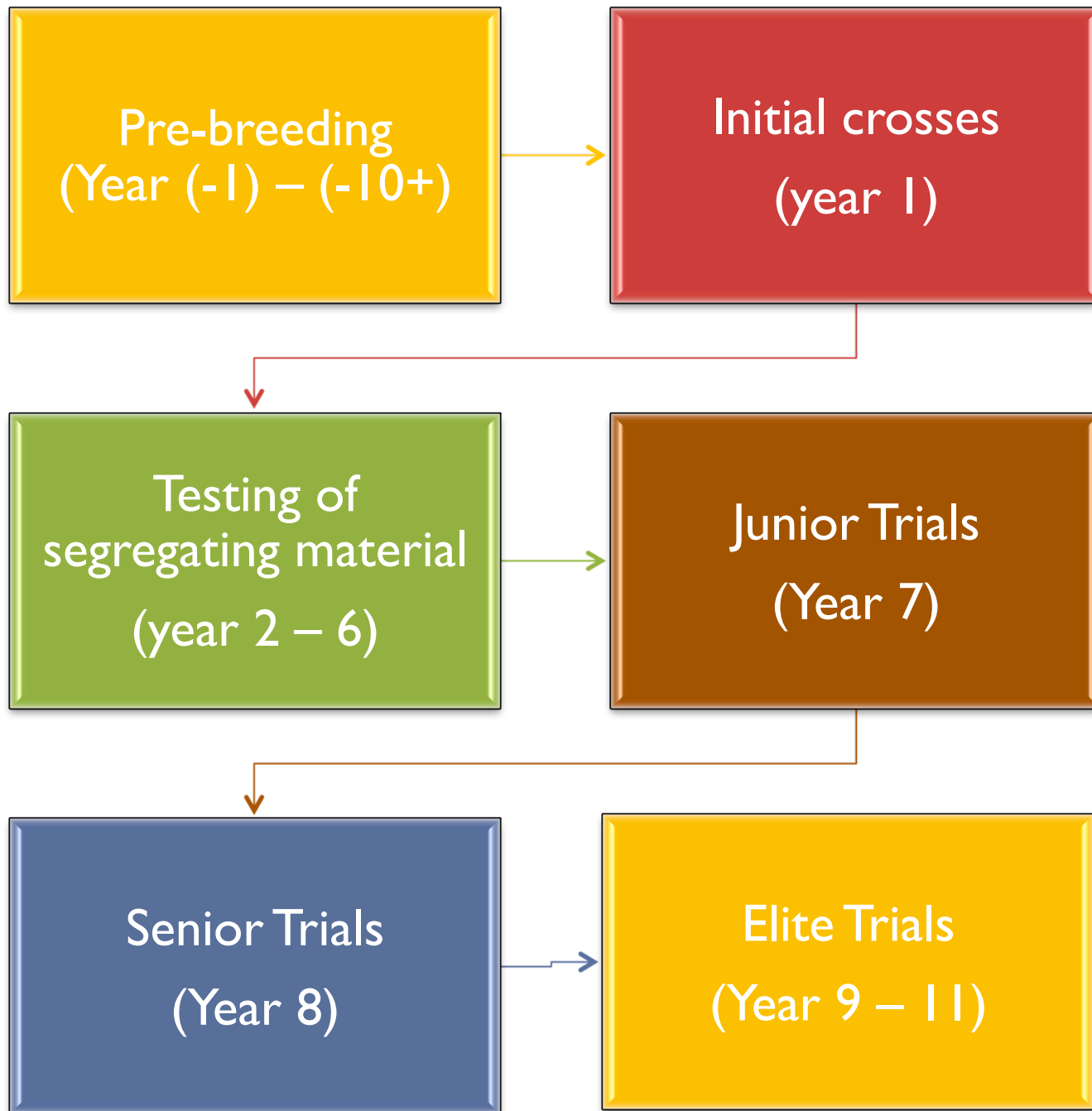
Introduction/



Stem rust	Avirulence genes	Virulence genes
UVPgt60	<i>Sr13, Sr14, Sr21, Sr22, Sr25, Sr26, Sr27, Sr29, Sr32, Sr33, Sr35, Sr36, Sr37, Sr39, Sr42, Sr43, Sr44, SrEm, SrTmp and SrSatu</i>	<i>Sr5, Sr6, Sr7b, Sr8a, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr16, Sr17, Sr24, Sr30, Sr31, Sr34, Sr38, Sr41 and SrMcN.</i>
Leaf rust	Avirulence genes	Virulence genes
UVPr9	<i>Lr2a, Lr2b, Lr3bg, Lr15, Lr16, Lr17 and Lr26</i>	<i>Lr1, Lr2c, Lr3a, Lr3ka, Lr10, Lr11, Lr14a, Lr20 and Lr30</i>
UVPr13	<i>Lr3a, Lr3bg, Lr3ka, Lr11, Lr16, Lr20 and Lr30</i>	<i>Lr1, Lr2a, Lr2b, Lr2c, Lr10, Lr14a, Lr15, Lr17, Lr24 and Lr26</i>
Stripe rust	Avirulence genes	Virulence genes
6E22A-	<i>Yr1, Yr3a, Yr4a, Yr4b, Yr5, Yr9, Yr10, Yr15, Yr27, YrA, YrCle, YrCv, YrHVII, YrMor, YrSd, YrSp and YrSu</i>	<i>Yr2, Yr6, Yr7, Yr8, Yr17 and Yr25</i>









Pre-breeding  
(Year (-1) – (-10+))

# Overview

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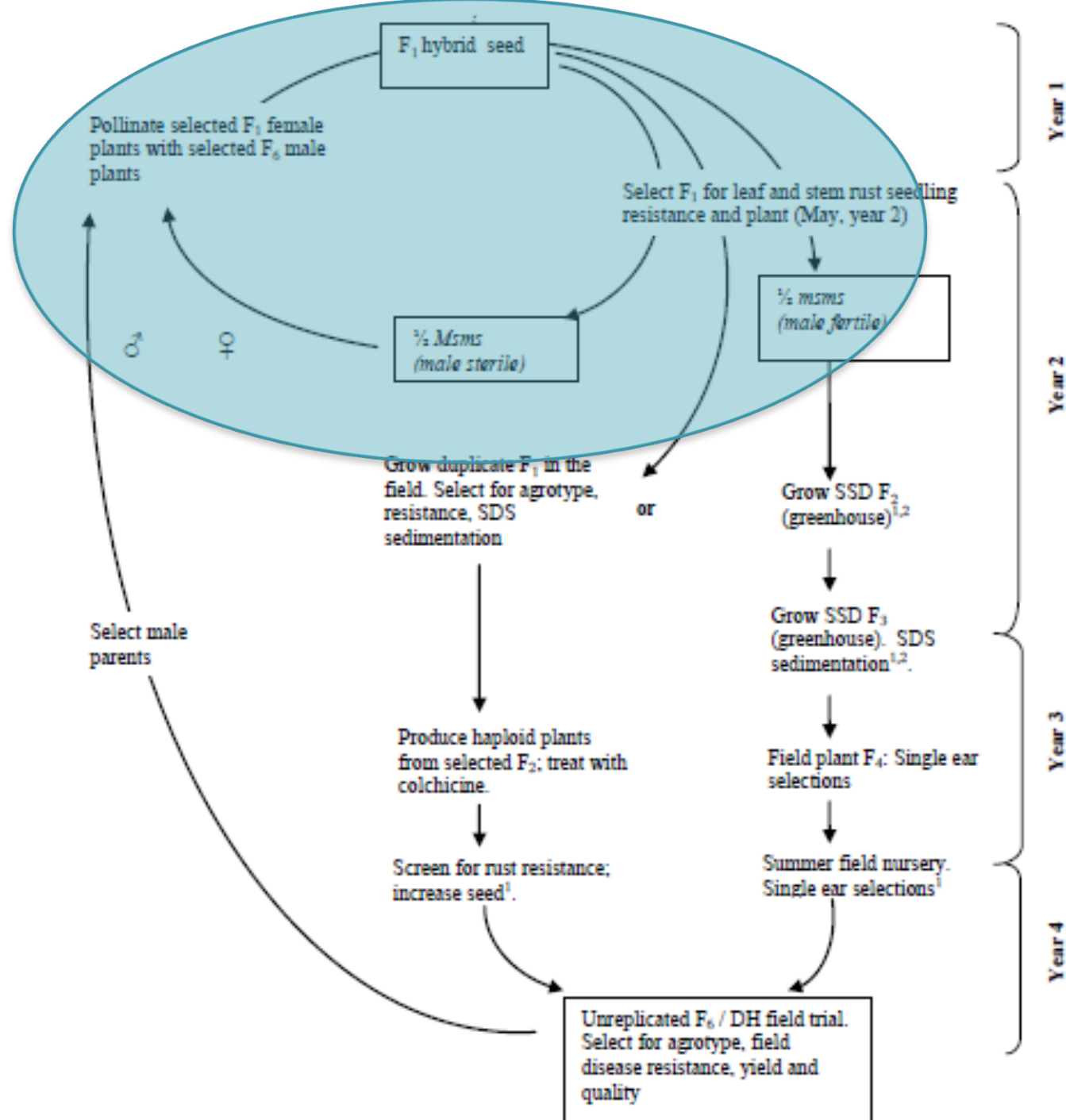
- Introduction
- **Wheat pre-breeding**
  - **MS-MARS**
  - **Doubled haploids**
  - **MAS**
- Case study
- Current and future work

# Introduction

Wheat pre-breeding/



- MS-MARS
- Since 1998
- WCT funding
- Concentrate mainly on rust resistance
  - Specific genes via MAS
  - APR
- Distribute rust resistance material
  - Nurseries (since 2006)
  - Lines together with molecular info and phenotype info











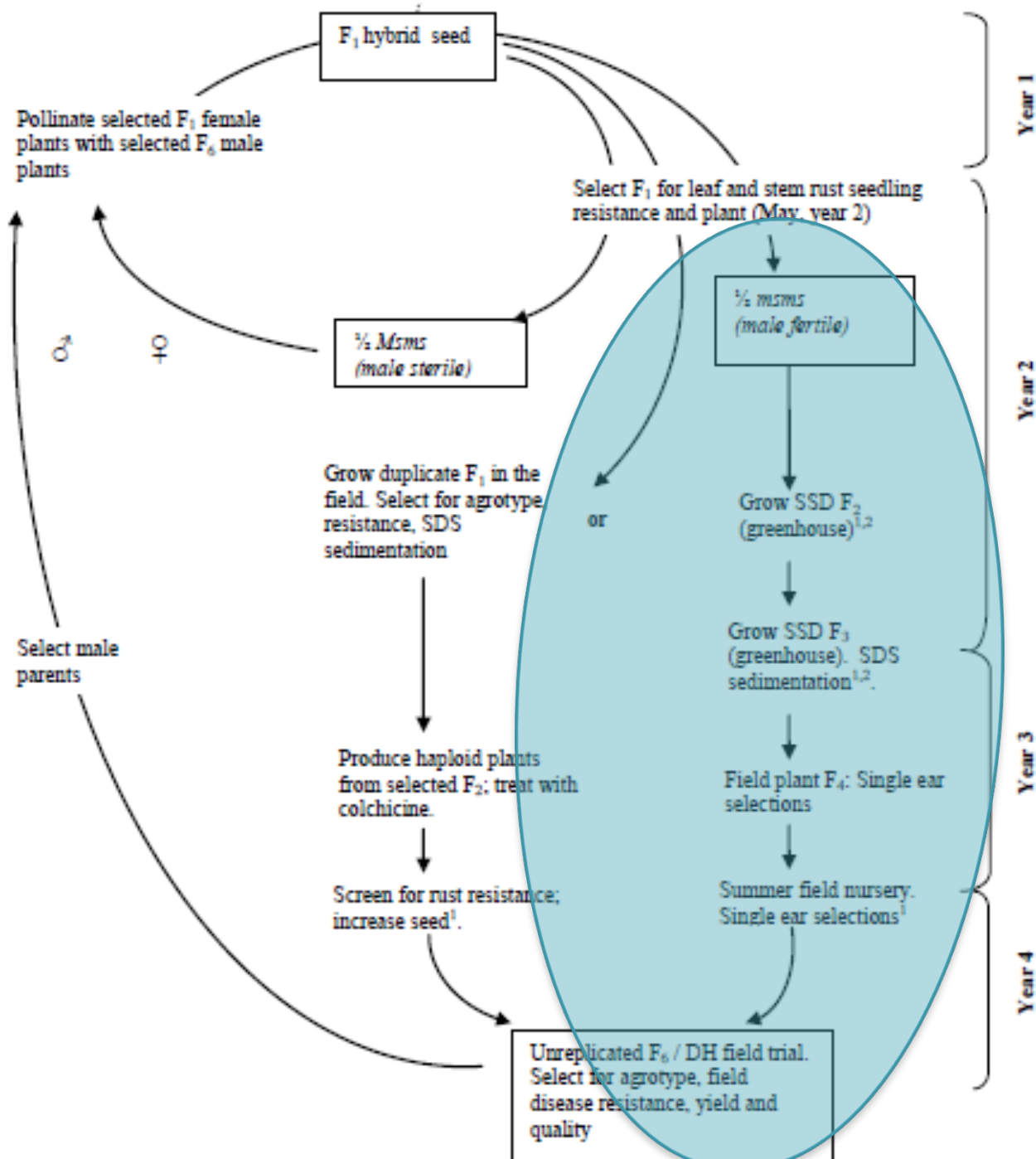














SU-PBL





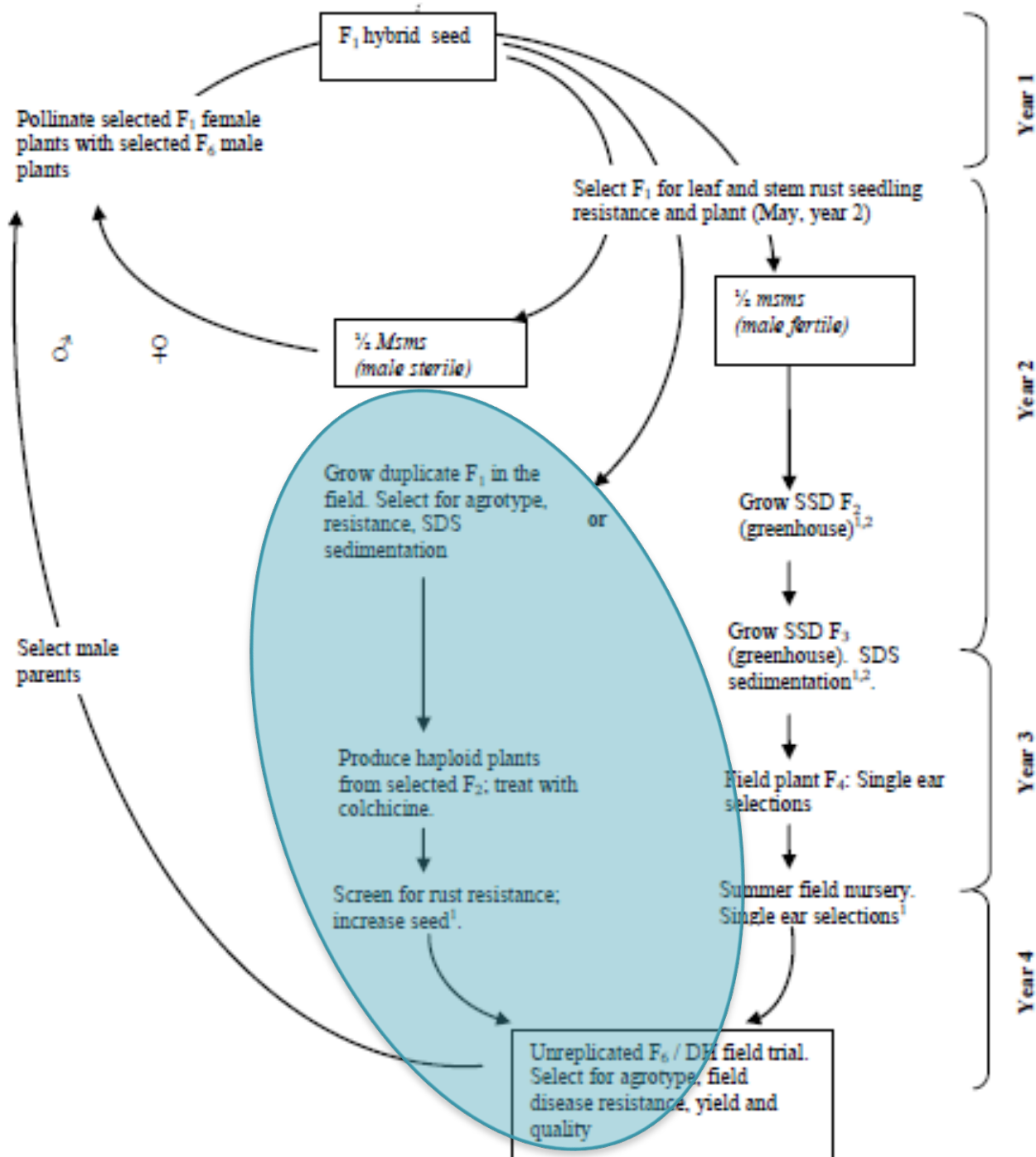














# Doubled haploids

Wheat pre-breeding/

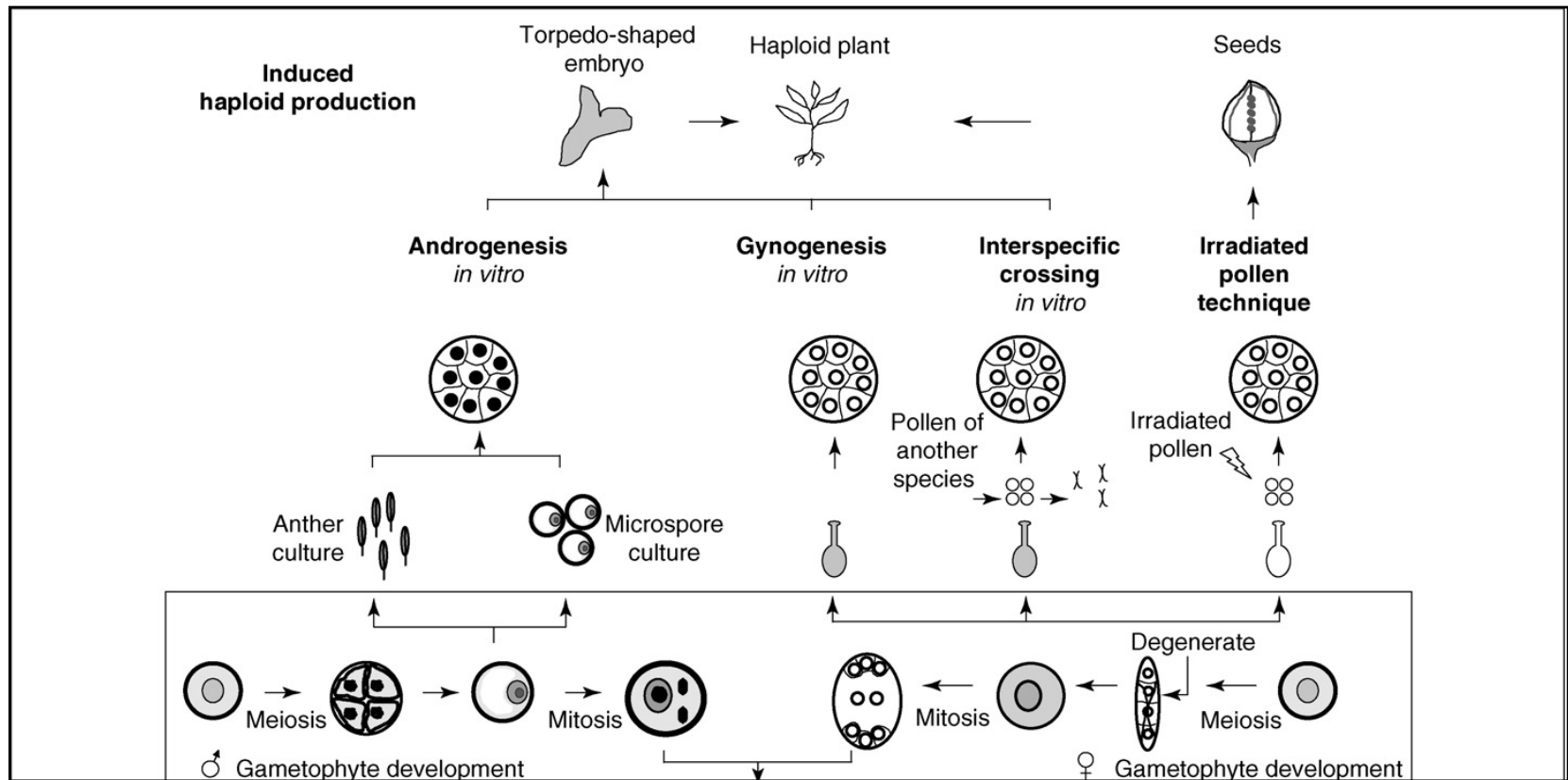


- Doubled haploid production is the most time efficient route to homozygosity in plants.
- There are three reasons for using this technology in SA context:
  - Most efficient route of fixing desirable traits into specific lines
  - Facilitates hybrid breeding
  - Doubled haploids can be applied in MAS
- There are three methods of doubled haploid production in wheat
  - Wide crosses
  - Anther culture
  - Microspore culture

# Doubled haploids

Wheat pre-breeding/

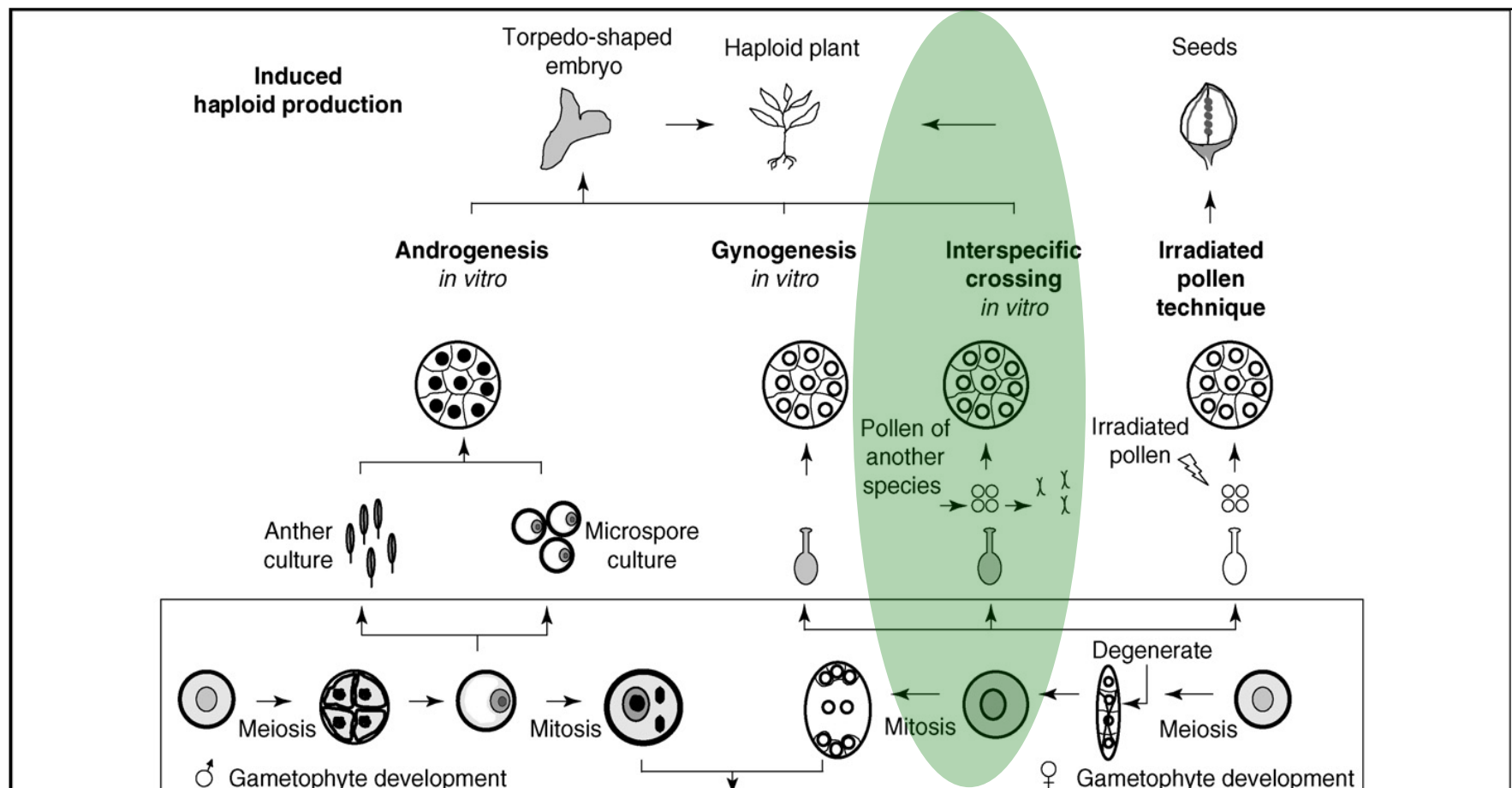
## Pathways for doubled haploid induction.



# Wide-crossing

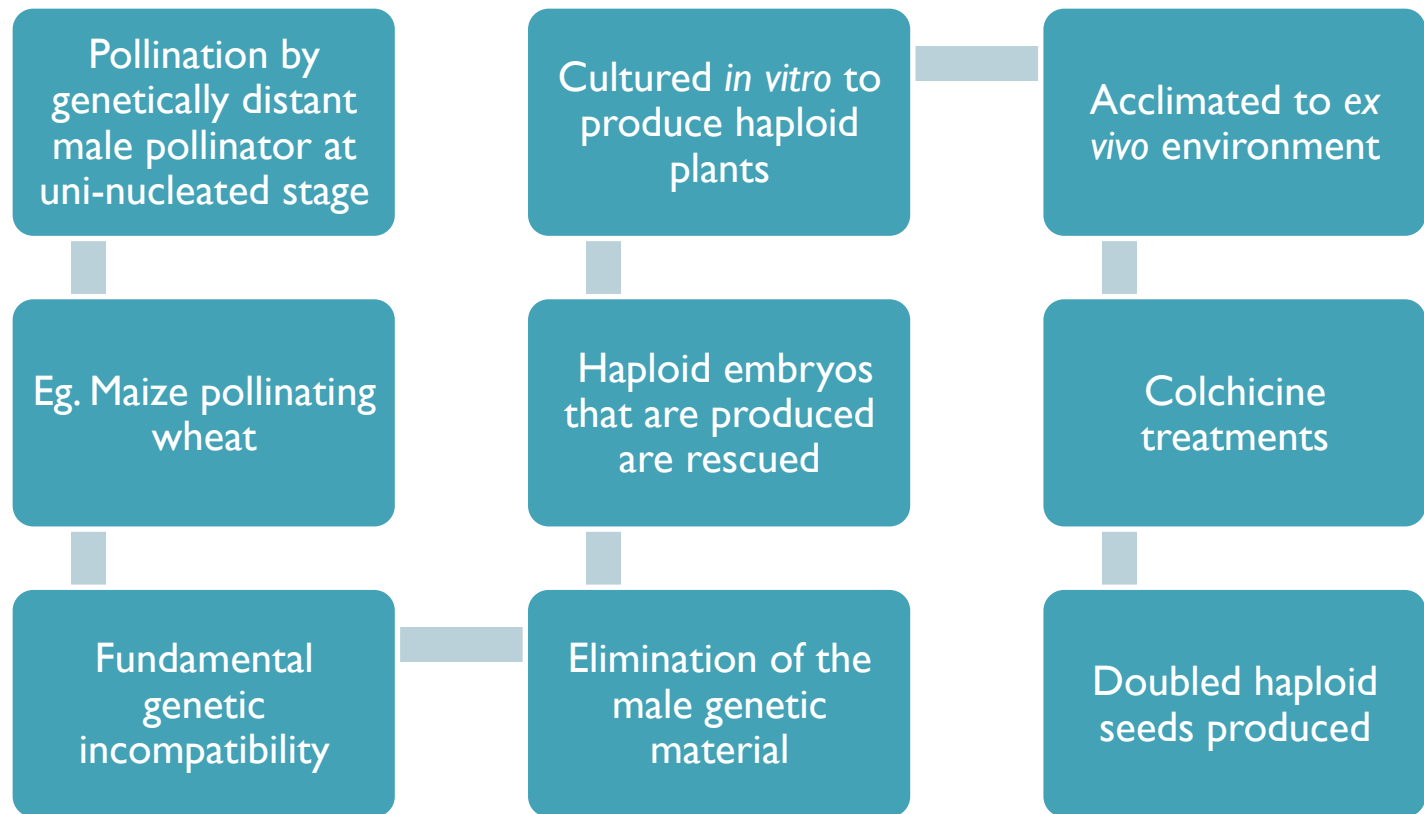
Wheat pre-breeding/Doubled haploids

## Pathways for doubled haploid induction.



# Wide-crossing

Wheat pre-breeding/Doubled haploids

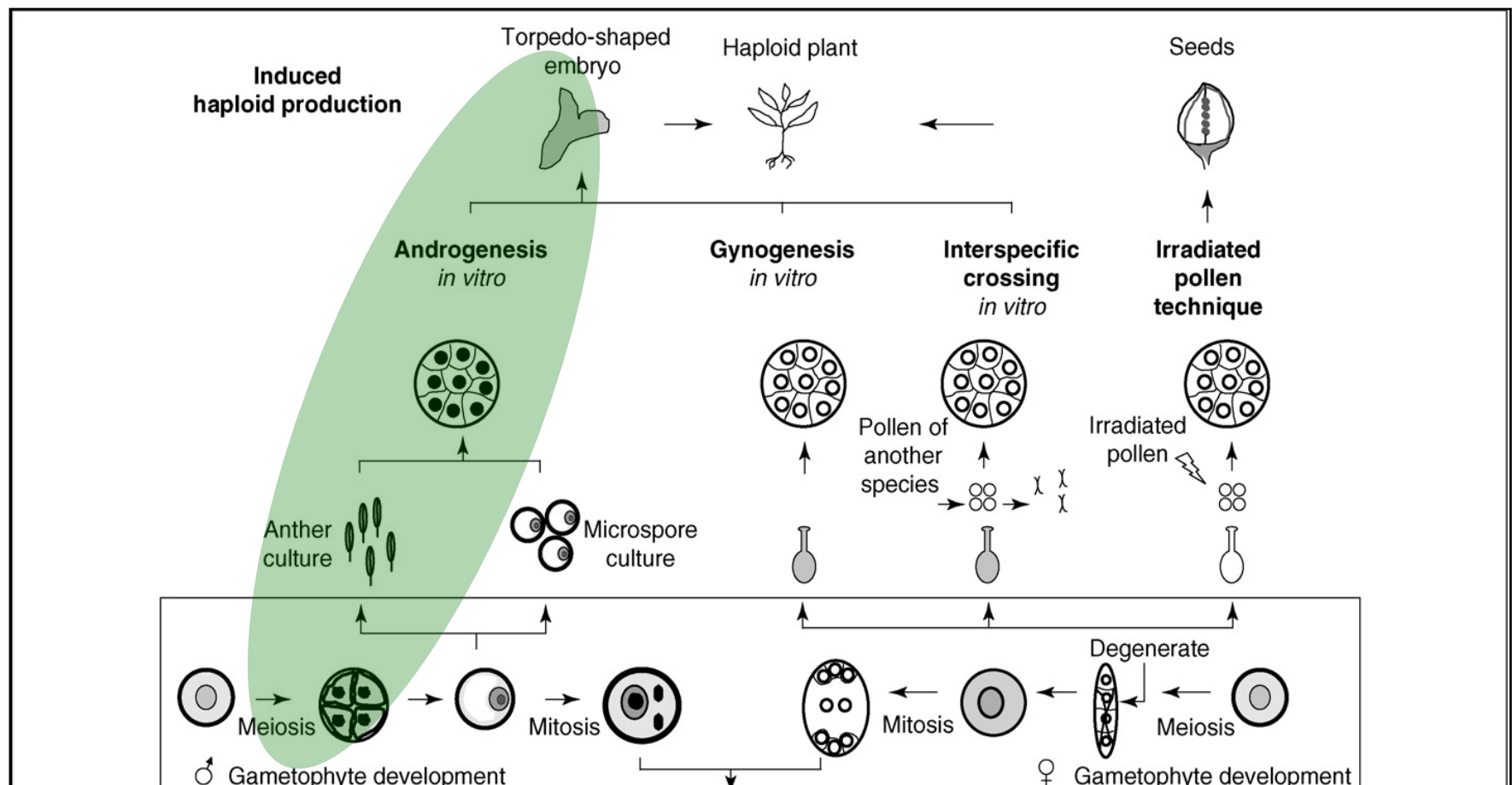




# Anther culture

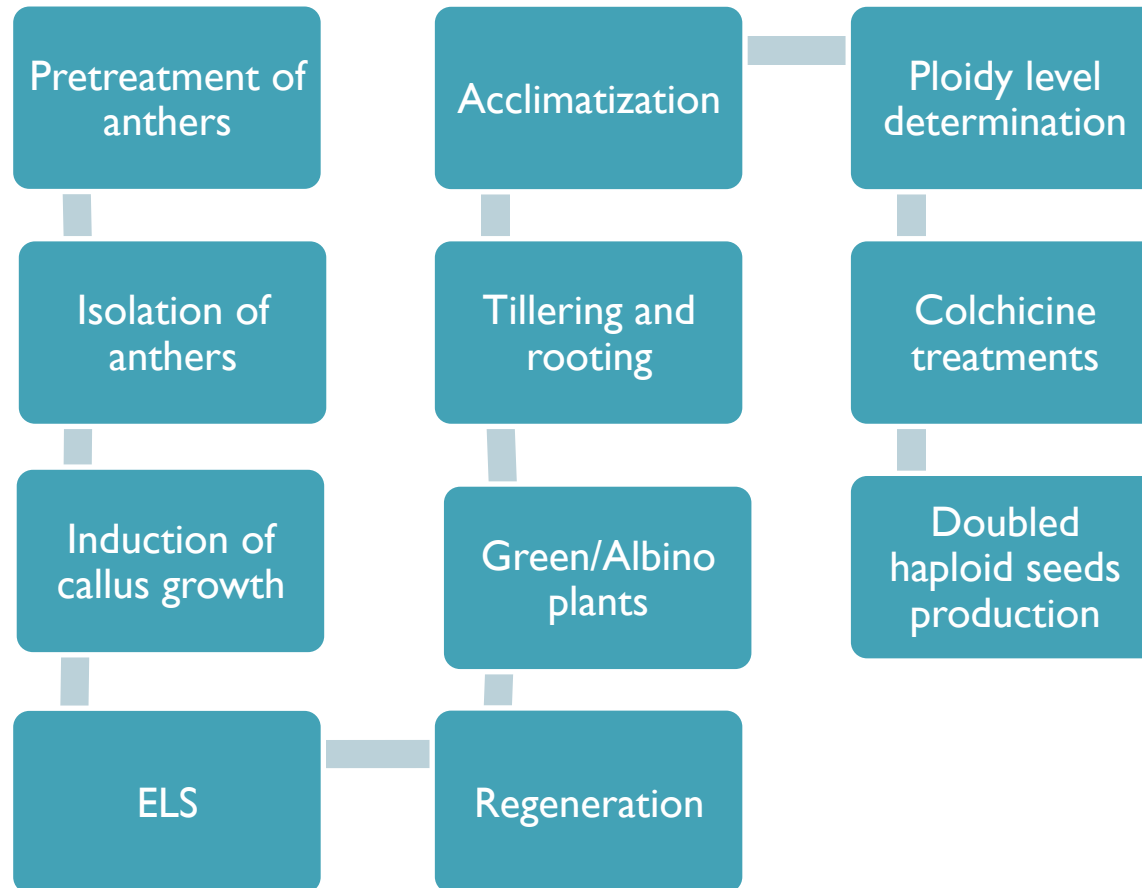
Wheat pre-breeding/Doubled haploids

## Pathways for doubled haploid induction.



# Anther culture

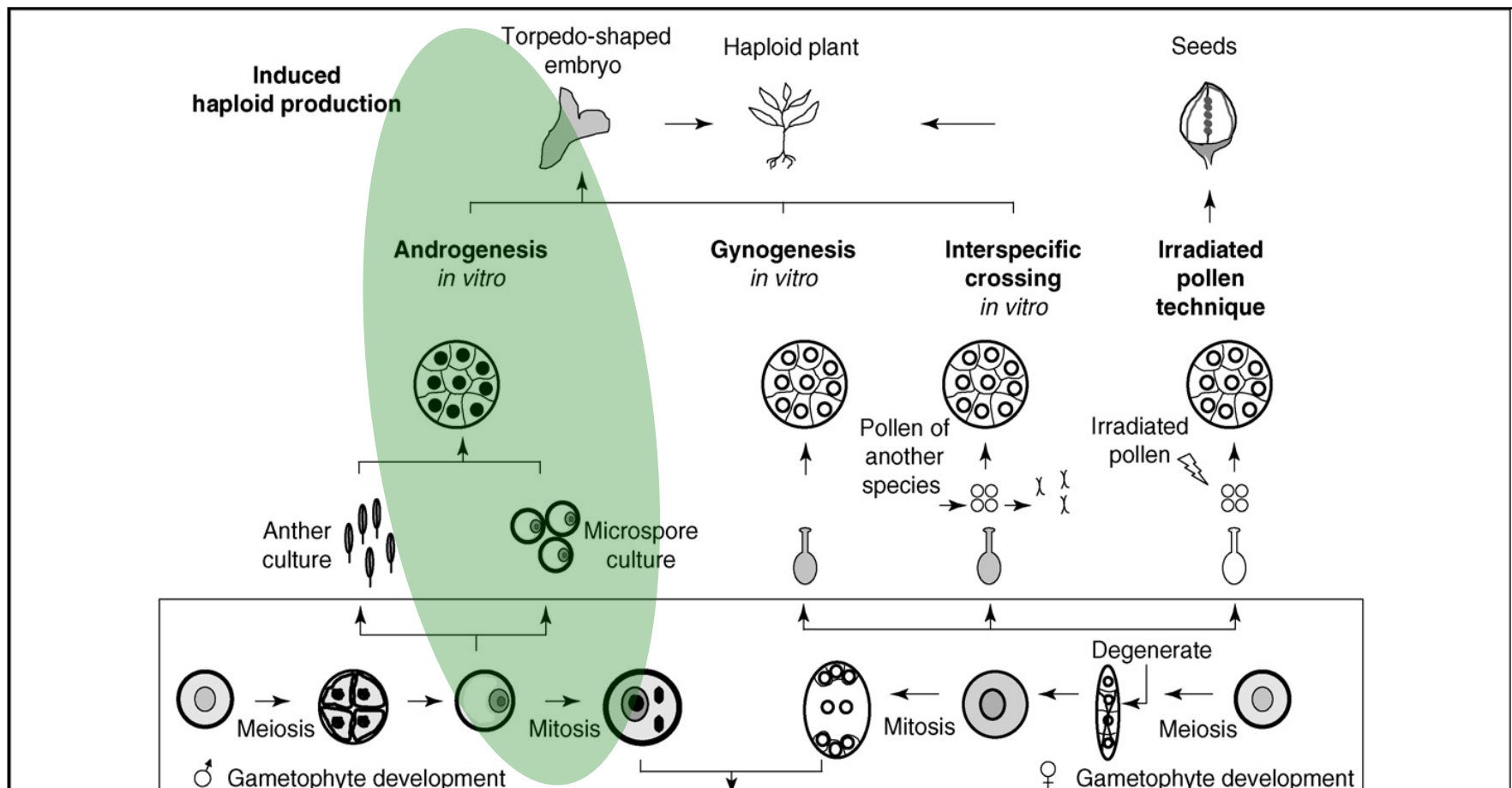
Wheat pre-breeding/Doubled haploids



# Microspores

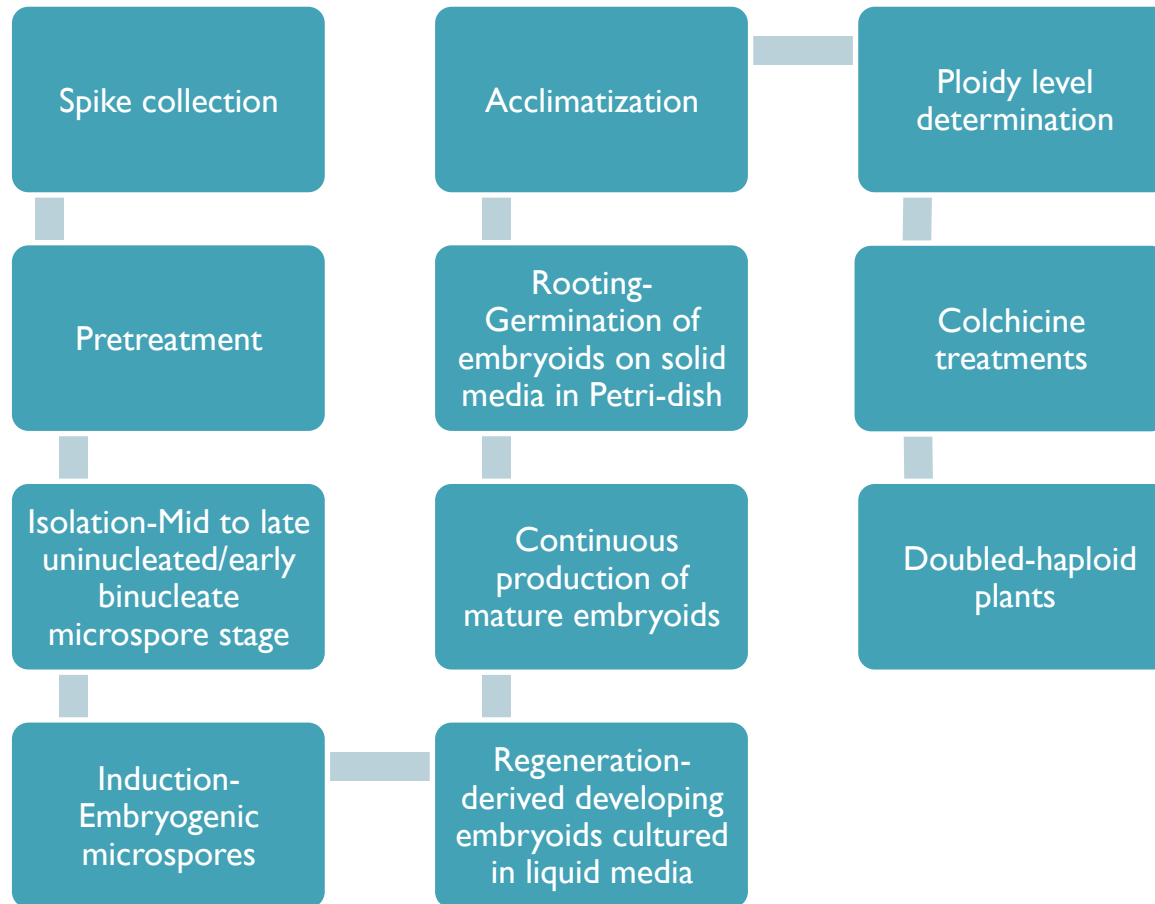
Wheat pre-breeding/Doubled haploids

## Pathways for doubled haploid induction.



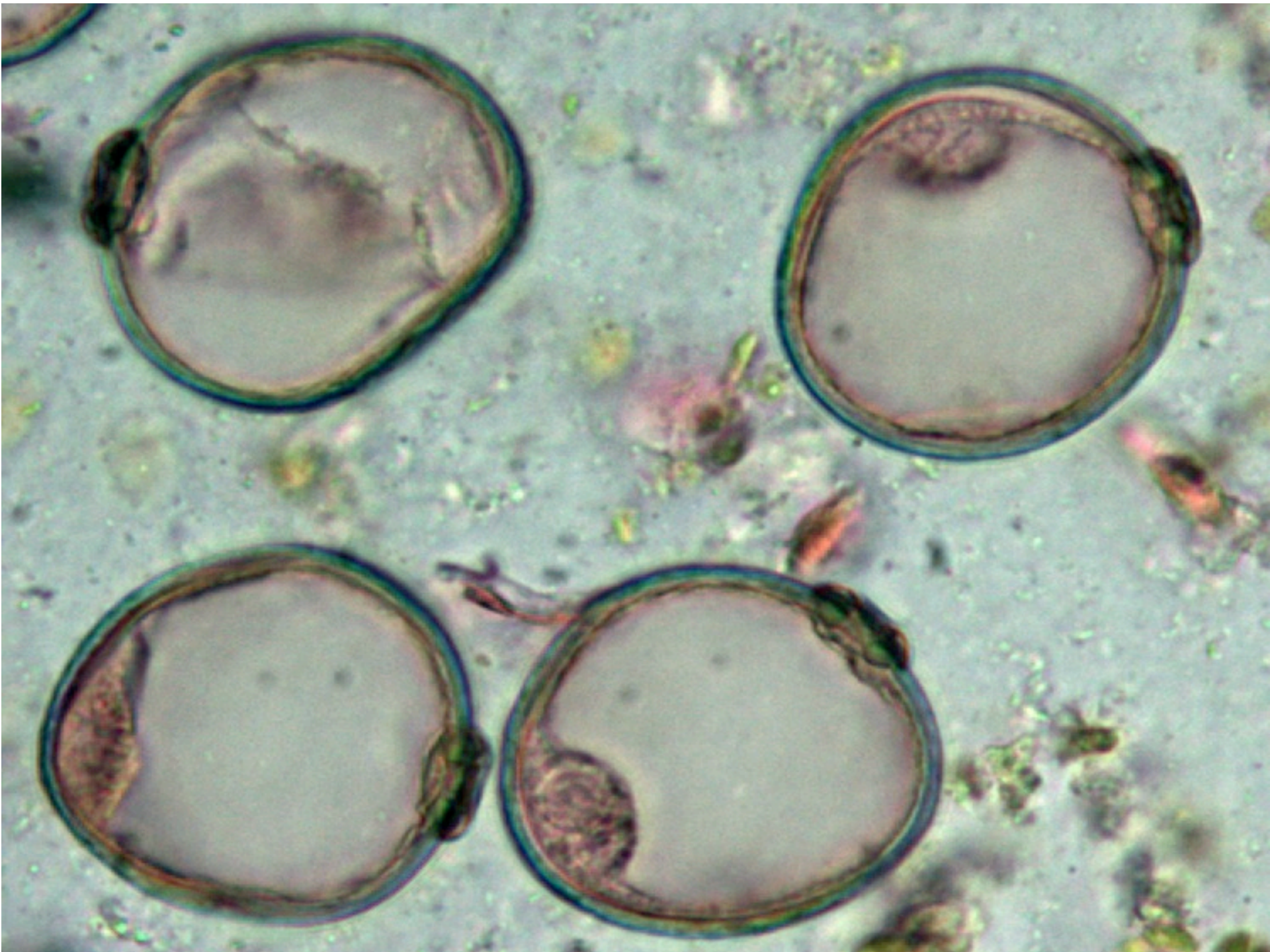
# Microspores

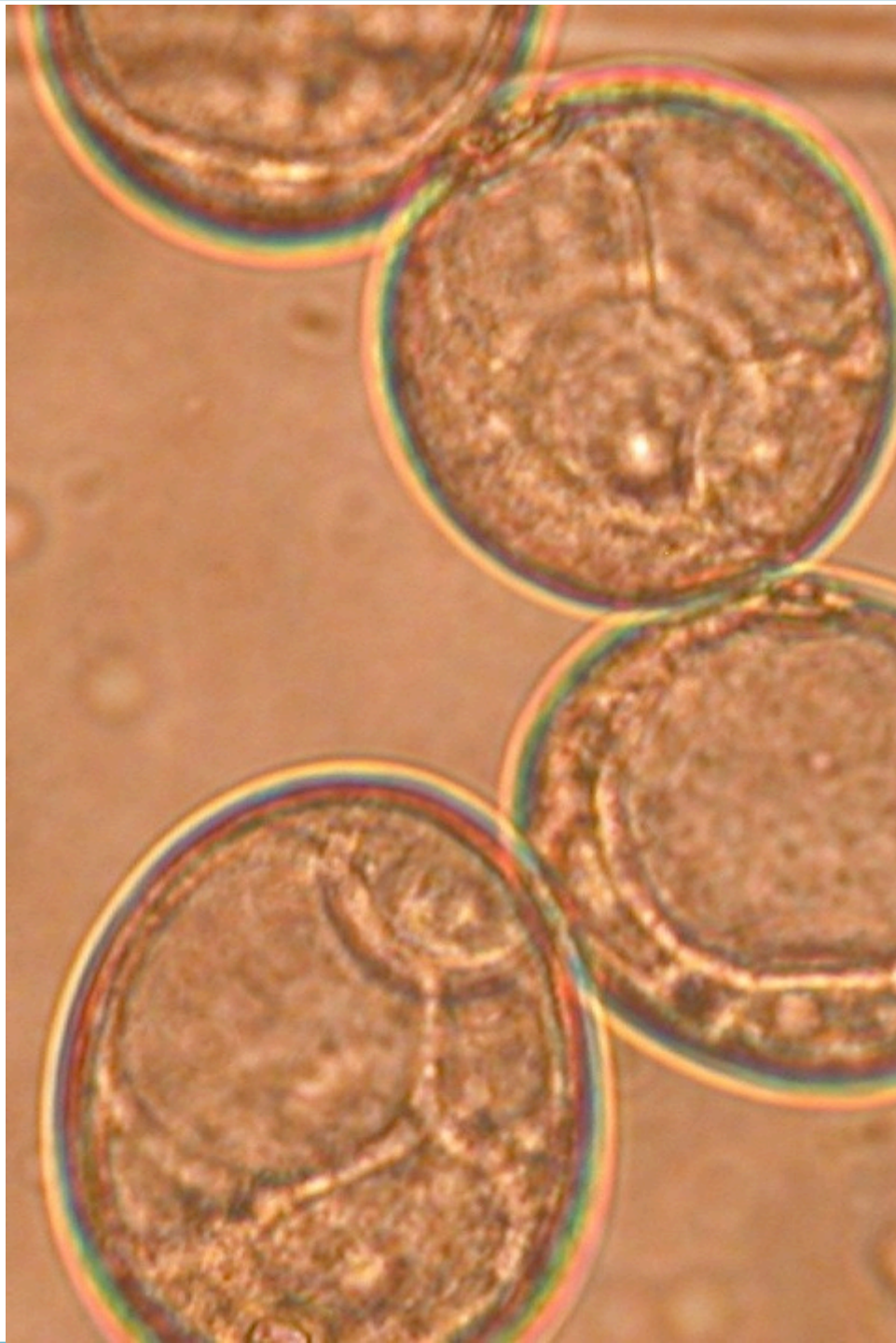
Wheat pre-breeding/Doubled haploids



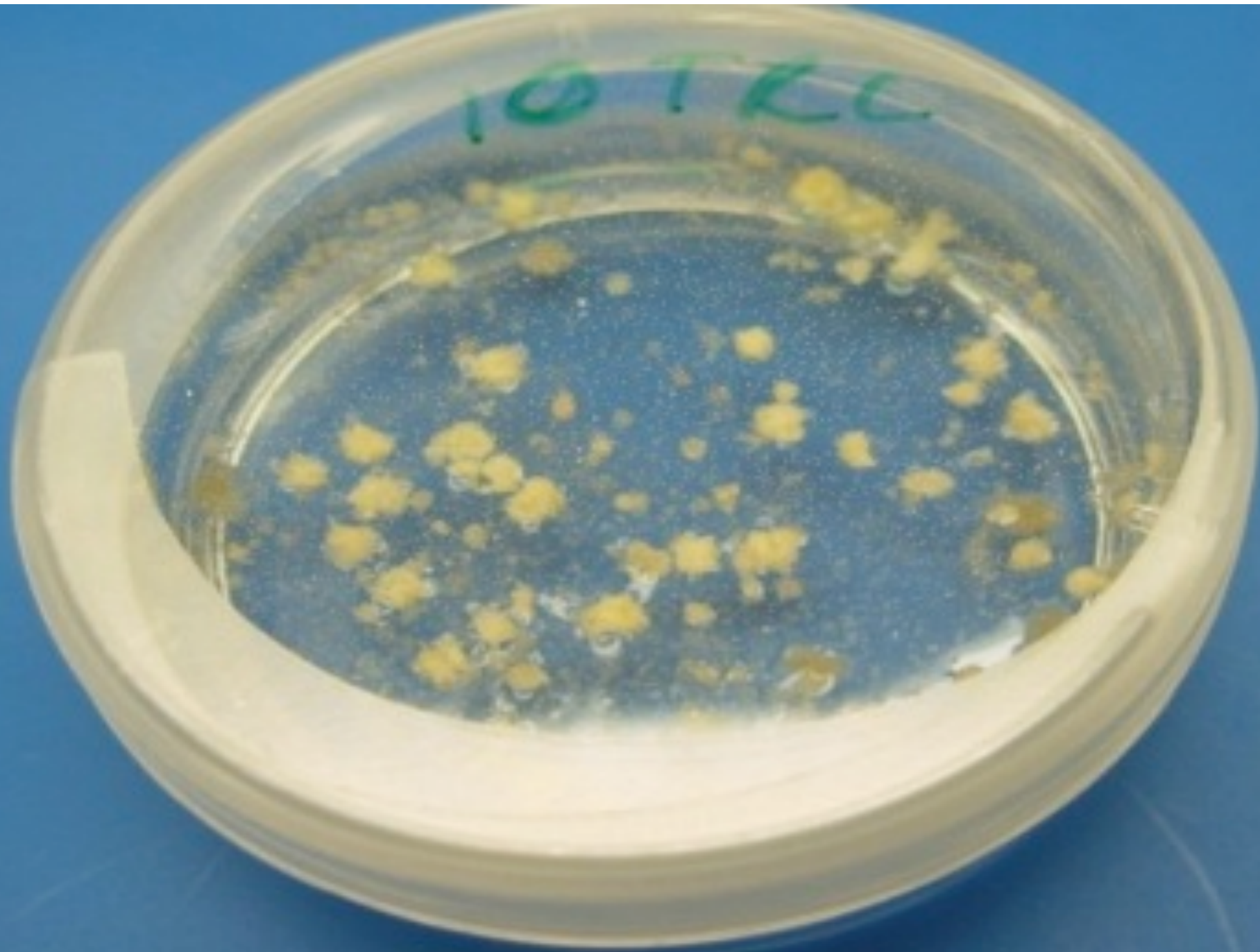


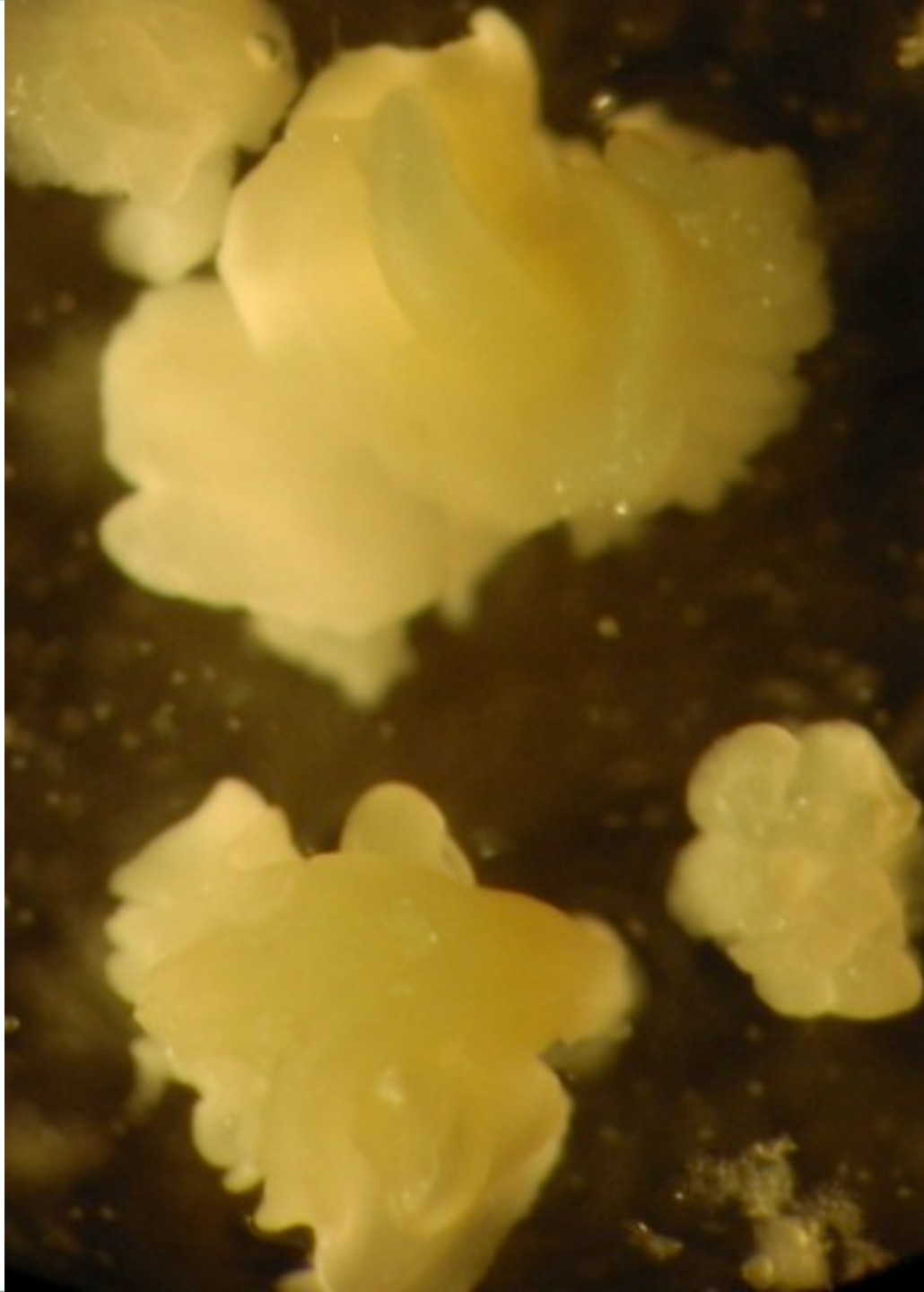


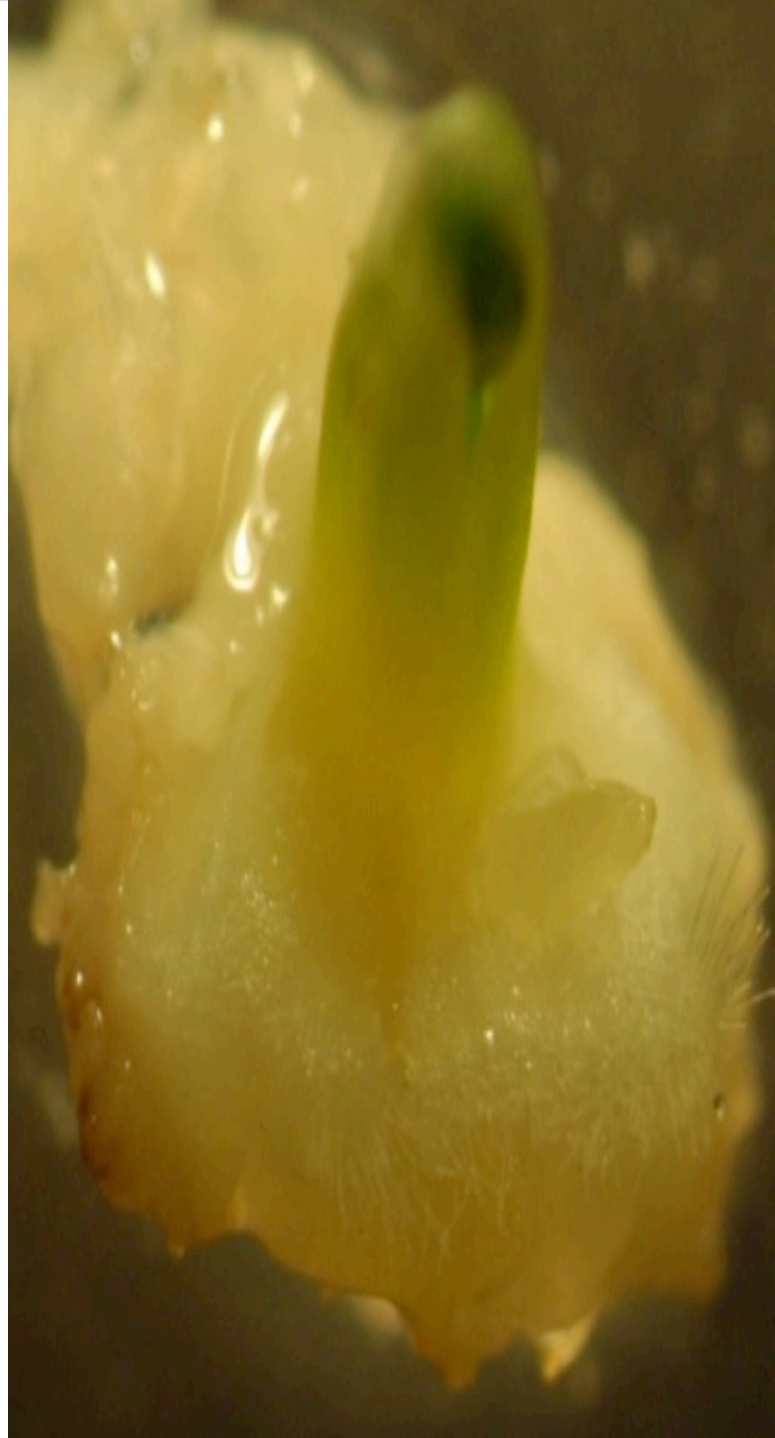






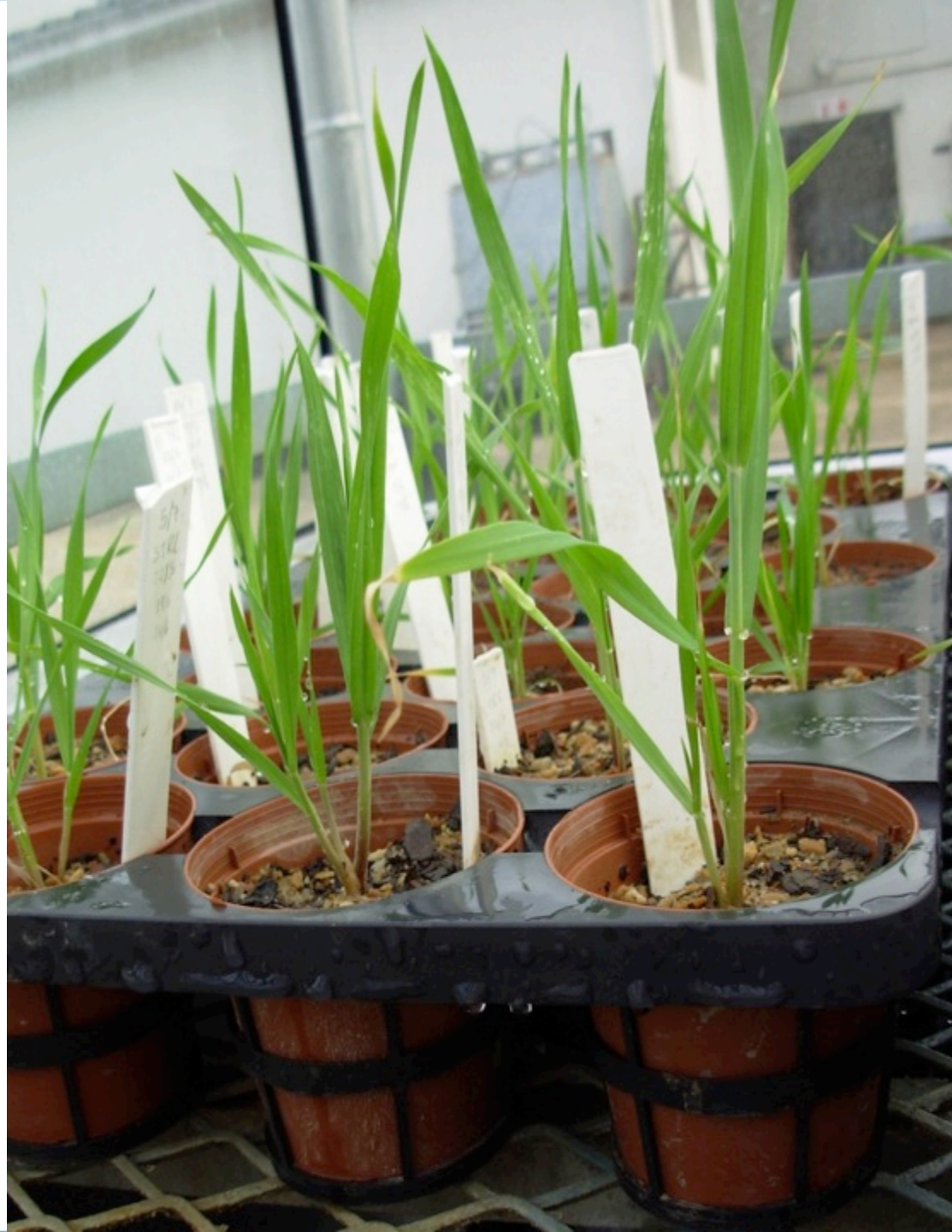














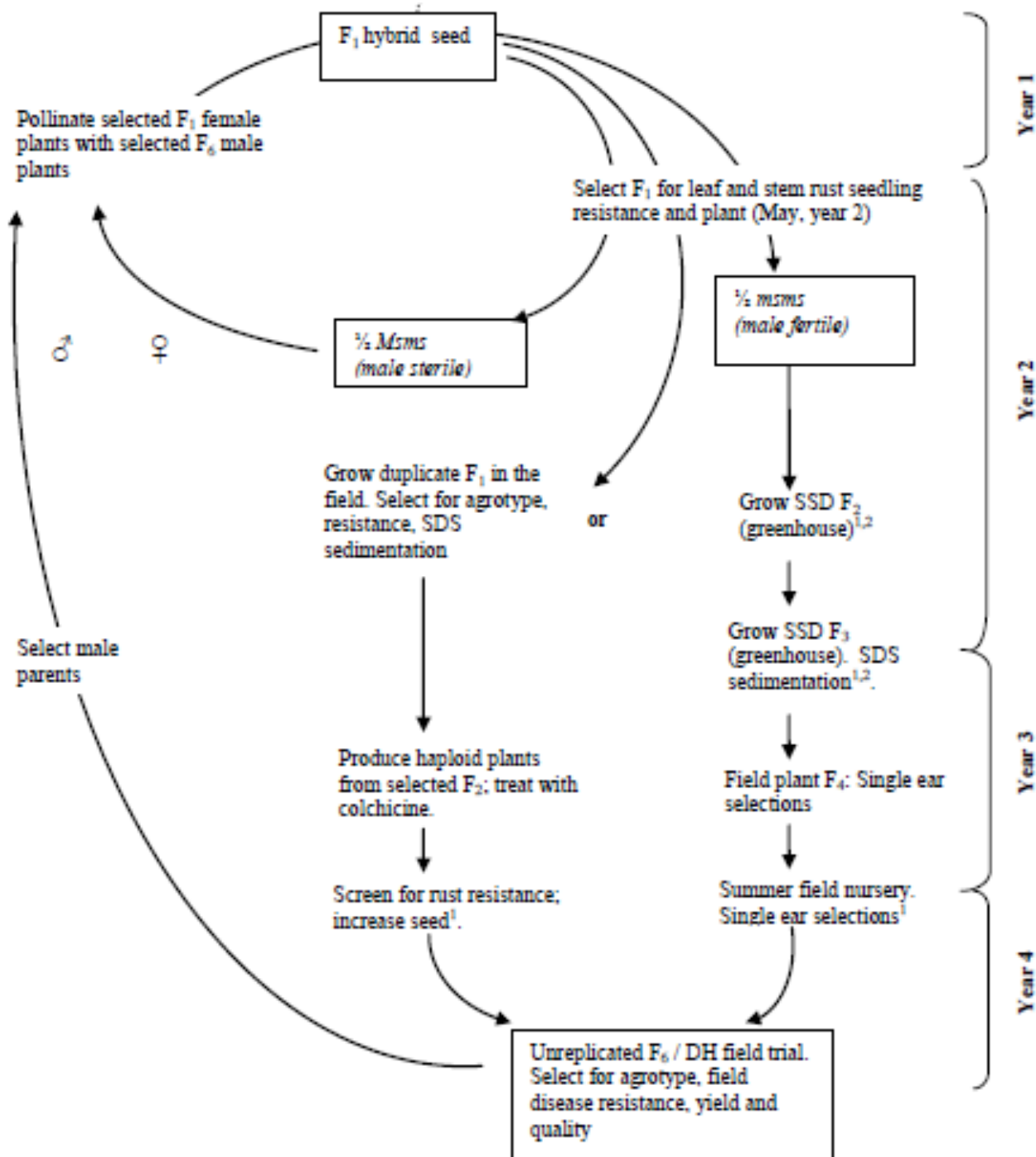
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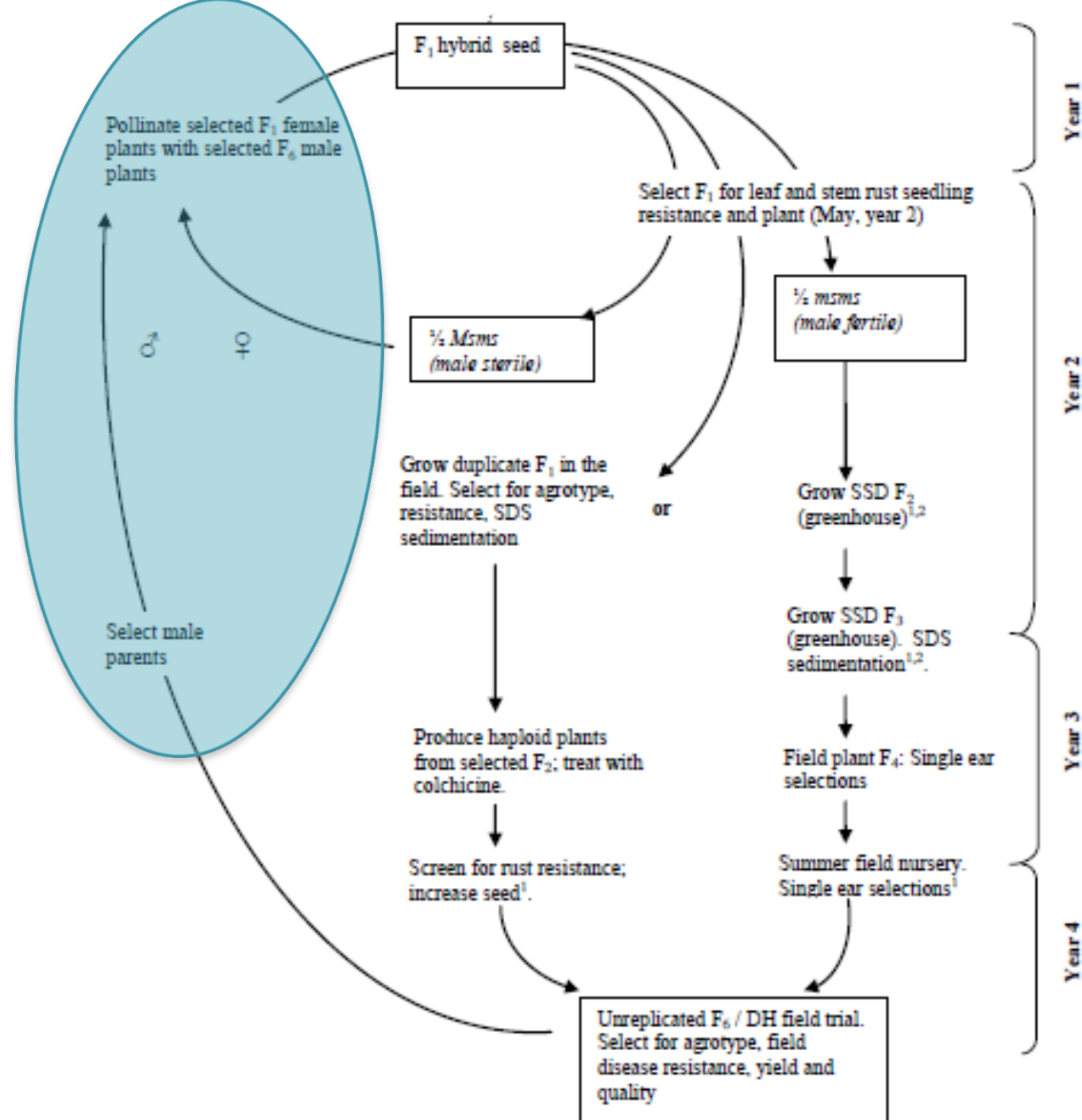


# Resistance genes & MAS

Wheat pre-breeding/



- *Lr24/Sr24*
  - Transferred from *Agropyron elongatum*
  - On chromosome 3D
  - Virulence in South Africa
- *Sr31/Lr26/Yr9*
  - Transferred from Rye
  - On chromosome 1B
  - Virulence in South Africa
- *Lr37/Sr38/Yr17*
  - Transferred from *Triticum ventricosum*
  - On chromosome 2AS
  - Virulence in South Africa
- *Sr2*
  - Transferred from tetraploid emmer wheat
  - On chromosome 3BL
  - Gene provides durable resistance to all stem rust pathotypes.
  - Need to be accompanied by other resistance genes to provide sufficient resistance
- *Lr34/Yr18*
  - On chromosome 7DS
  - Provides durable resistance to all leaf and stripe rust pathotypes.
  - Need to be accompanied by other resistance genes to provide sufficient resistance



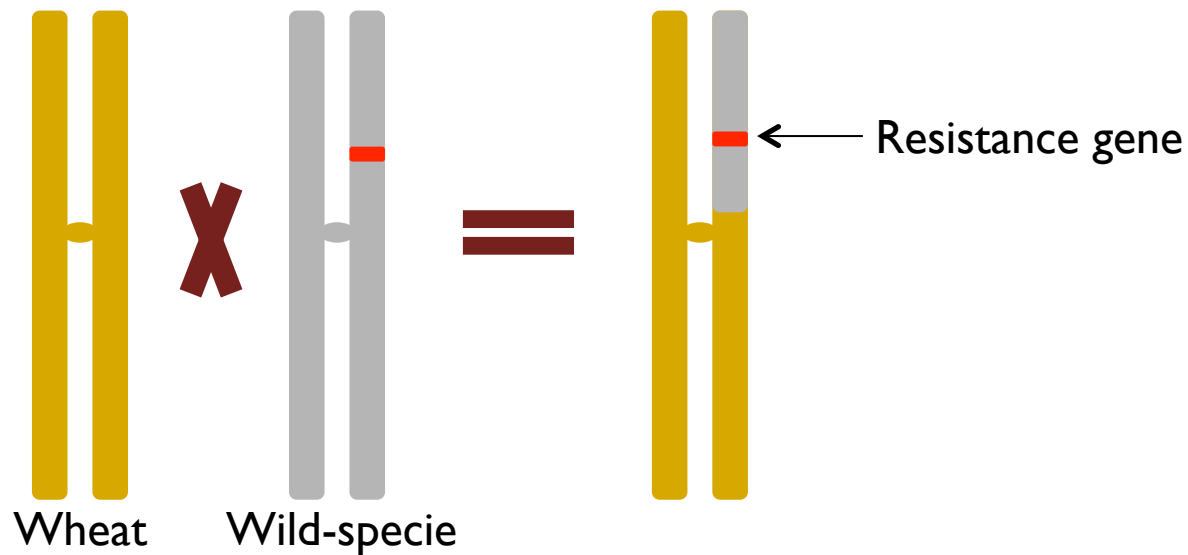


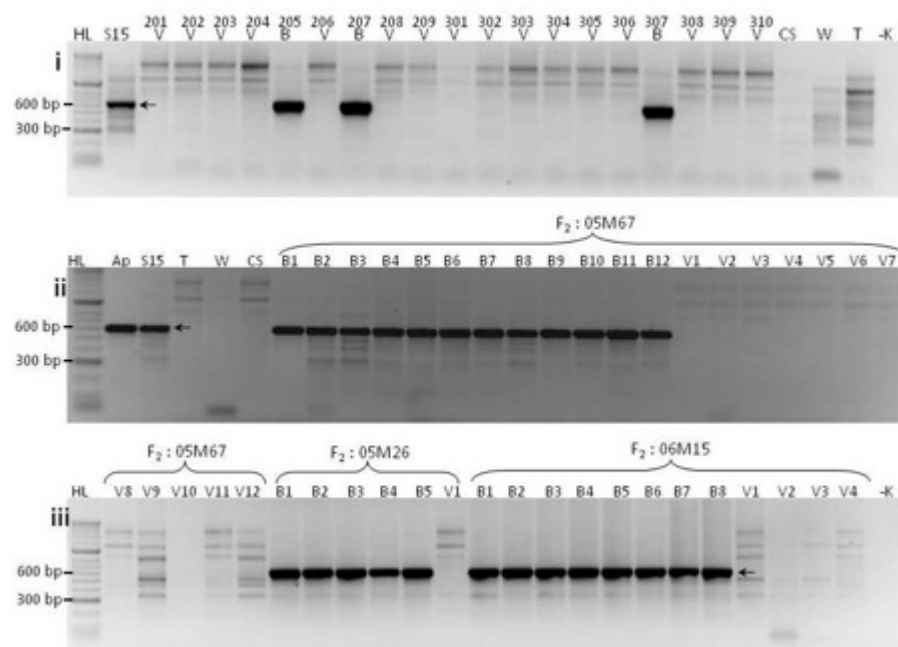
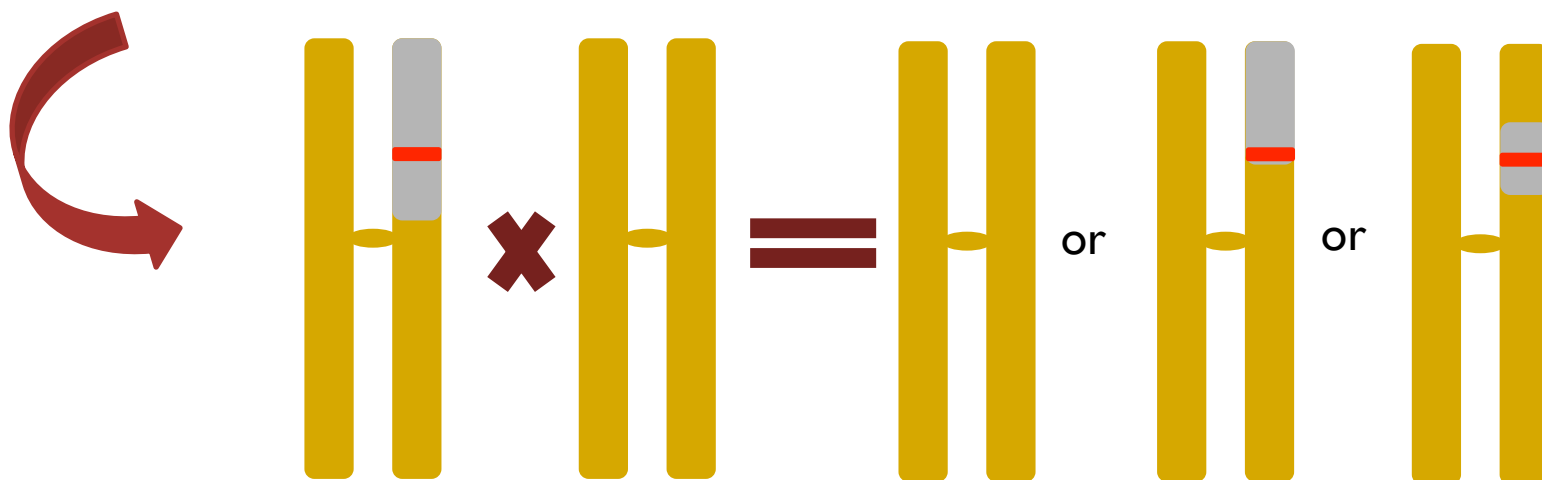
# Novel Wild-species Resistance sources

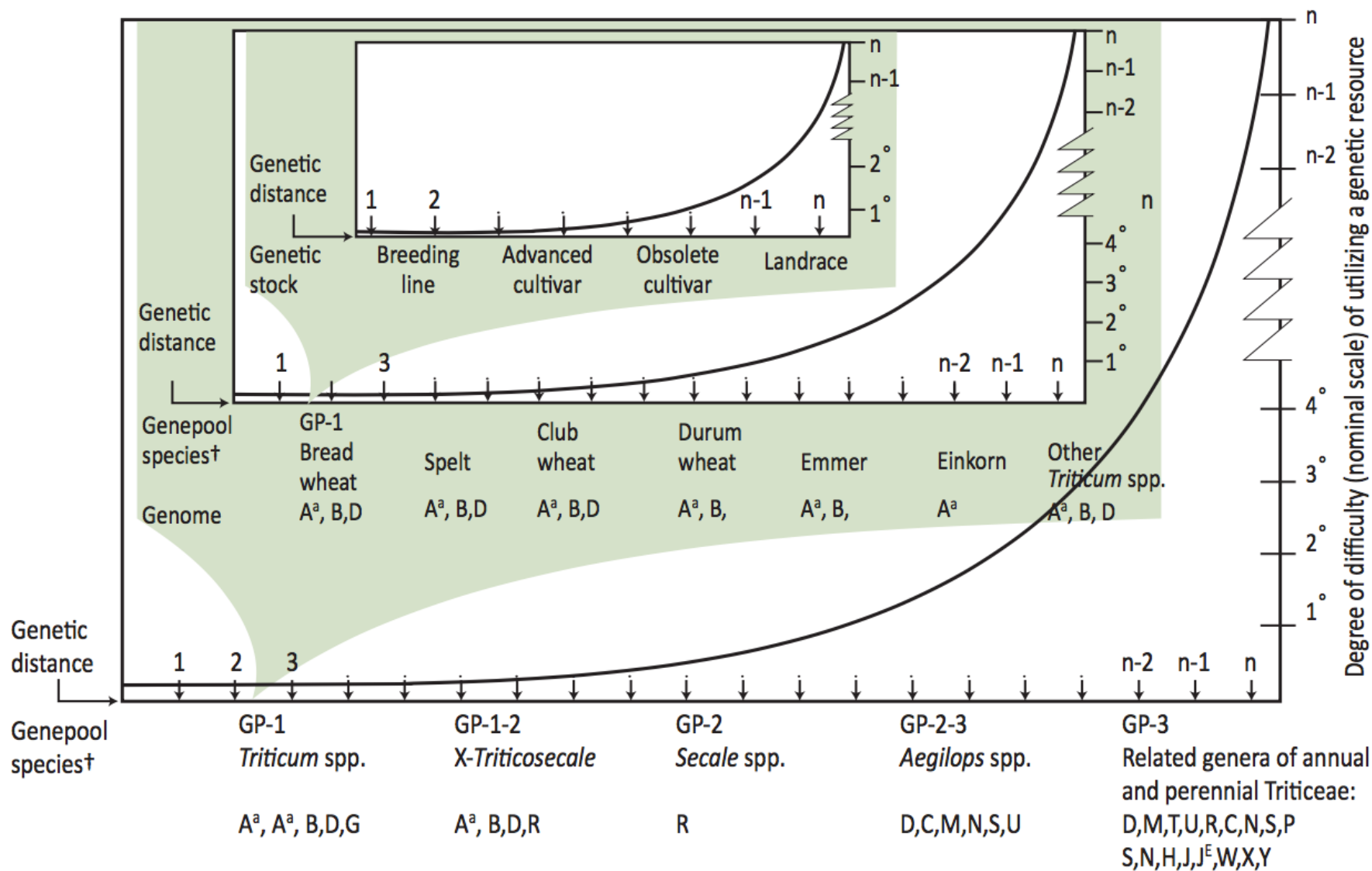
Wheat pre-breeding/



- Wild-species good source of novel resistance genes
- Transfer between wheat and wild-specie relatives possible









# *T. kotschyi*: Lr54/Yr37 (2DL)

Wheat pre-breeding/Novel Wild-species Resistance sources



# *T. sharonense*: Lr56/Yr38 (6A)

Wheat pre-breeding/Novel Wild-species Resistance sources



# *T. peregrinum* (UUSS): Lr59

Wheat pre-breeding/Novel Wild-species Resistance sources





# *T. neglecta*: Lr62/Yr42 (6A)

Wheat pre-breeding/Novel Wild-species Resistance sources



# MAS

## Wheat pre-breeding/Novel Wild-species Resistance sources

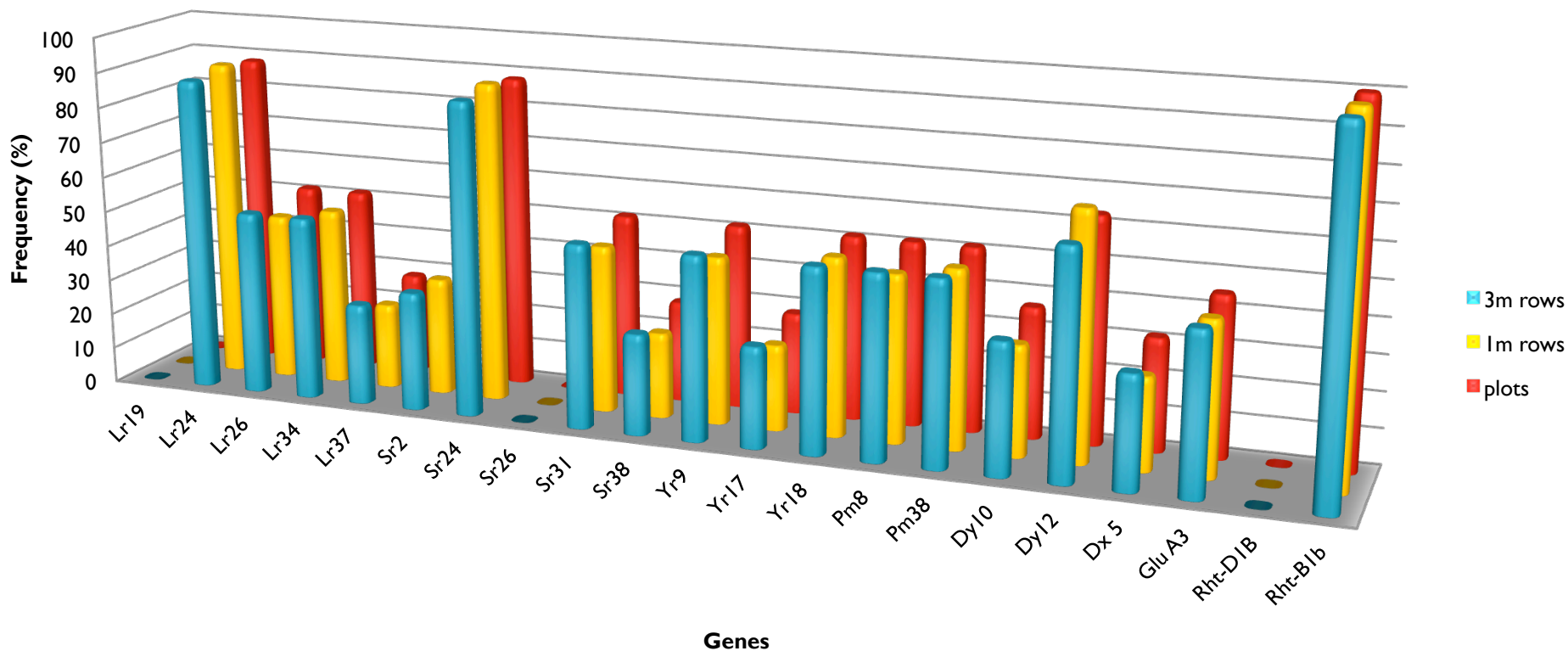


Resistance translocation	Primer	Primer sequence	Ta (°C)	Amplified fragment size (bp)
<i>Lr53/Yr35</i>	S8N1-OF	5'-CACGTTGGTAACTGAACATT-3'	48	500
	S8N1-OR	5'-CTCACGTTGGACTTAAA-3'		
<i>Lr54/Yr37</i>	S14 275F	5'-CATGCAGAAAACGACACACC-3'	60	297
	S14 252R	5'-GGTAAGTGGTCAGGCGTTGT-3'		
<i>Lr56/Yr38</i>	S8N1-OF	5'-CACGTTGGTAACTGAACATT-3'	48	500
	S8N1-OR	5'-CTCACGTTGGACTTAAA-3'		
<i>Lr59</i>	S15 T3F	5'-GTCACCTTGCTTGAATTTAATG-3'	52	622
	S15 T3R	5'-TCCATAGCTGGTAGCTAGATG-3'		
<i>Lr62/Yr42</i>	Opw 7.2F	5'-CAGGAGCATAGTCATACTTGGG-3'	60	700
	Opw 7.2R	5'-CTGGACGTCAACAATGGC-3'		

# Distribution of resistant material

Wheat pre-breeding/

## Gene frequencies – 2014 Nursery



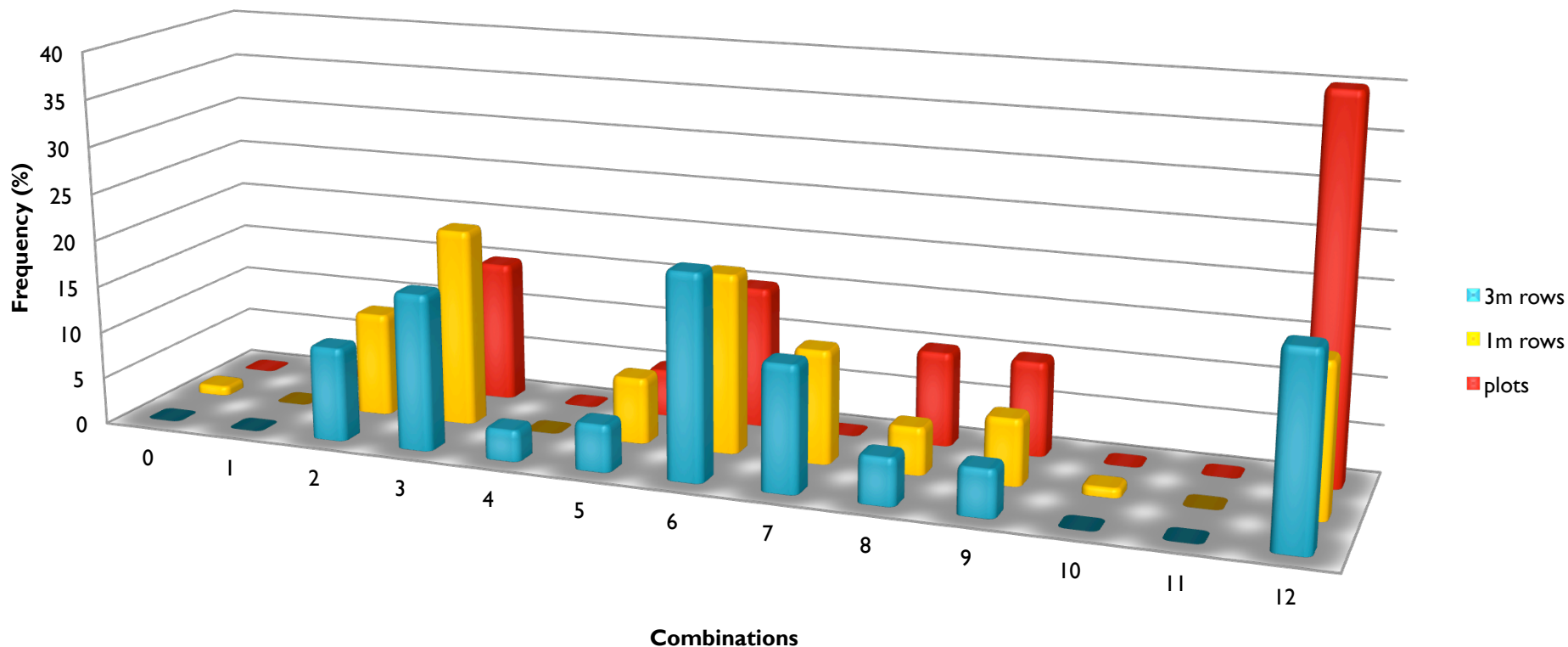


# Distribution of resistant material

Wheat pre-breeding/



## Gene combinations – 2014 Nursery



# Distribution of resistant material

Wheat pre-breeding/



8th SU-PBL Nursery Molecular Data (2013)																					
Entries	Disease Resistance															Quality				Height	
	Lr19	Lr24	Lr26	Lr34	Lr37	Sr2	Sr24	Sr26	Sr31	Sr38	Yr9	Yr17	Yr18	Pm8	Pm38	Dy10	Dy12	Dx 5	Glu A3	Rht-D1B	Rht-B1b
13US1M001	0	1	0	1	0	1	1	0	0	0	0	0	1	0	1	1	0	1	0	0	1
13US1M002	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1
13US1M003	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	0	1
13US1M004	0	1	0	1	0	1	1	0	0	0	0	0	1	0	1	0	1	0	0	0	1
13US1M005	0	0	1	1	0	0	0	0	1	0	1	0	1	1	1	0	1	0	1	0	1
13US1M006	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1
13US1M007	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1
13US1M008	0	1	1	0	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1
13US1M009	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	1	0	1
13US1M010	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1
13US1M011	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0	1
13US1M012	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1
13US1M013	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1
13US1M014	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0	1	0	0	0	1
13US1M015	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0	1	0	0	0	1
13US1M016	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	0	1
13US1M017	0	1	1	0	0	0	1	0	1	0	1	0	0	1	0	1	0	1	1	0	1
13US1M018	0	1	1	1	0	0	1	0	1	0	1	0	1	1	1	0	1	0	1	0	1
13US1M019	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0	1	0	1	0	1
13US1M020	0	1	1	0	0	0	1	0	1	0	1	0	0	1	0	1	0	1	0	0	1

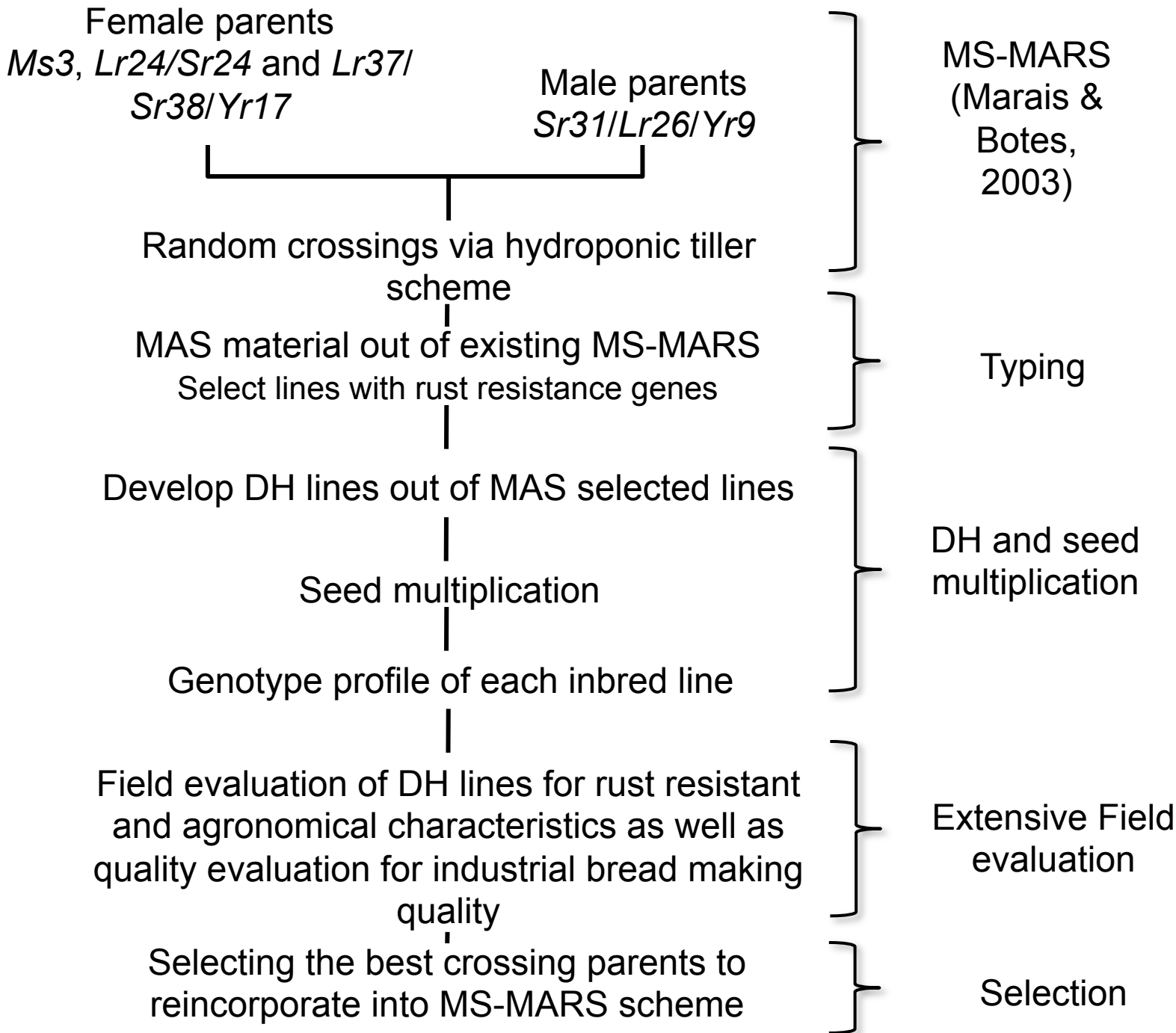
# Overview

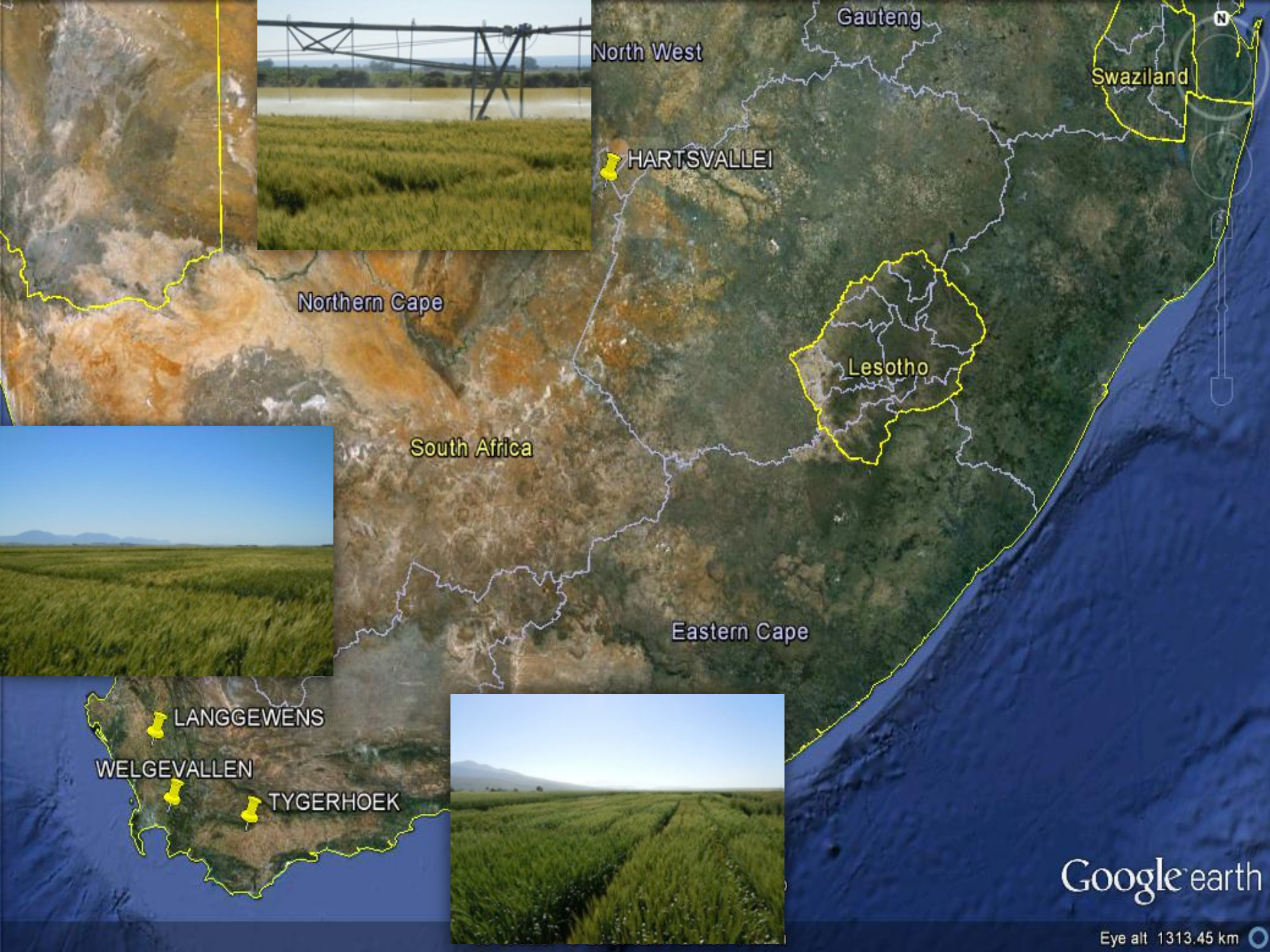
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- Introduction
- Wheat pre-breeding
- **Case study**
  - **MAS**
  - **DH and seed multiplication**
  - **Field evaluation**
    - **Baking quality evaluation**
    - **Rust screening**
    - **Data analysis**
- Current and future work







North West

Gauteng

Swaziland

HARTSVALLEI

Northern Cape

South Africa

Lesotho

Eastern Cape

LANGGEWENS

WELGEVALLEN

TYGERHOEK

Google earth

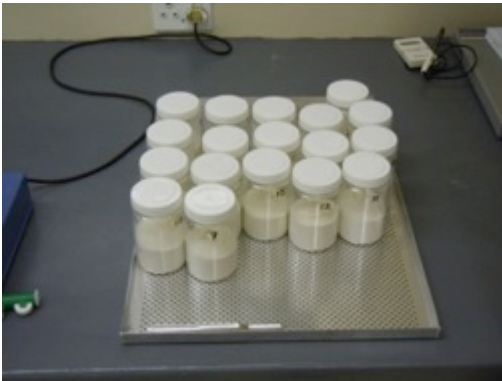
Eye alt 1313.45 km



# Quality evaluation

## Case Study/

### *Flour quality*



### *Rheological quality*



### *Baking quality*





# Quality evaluation

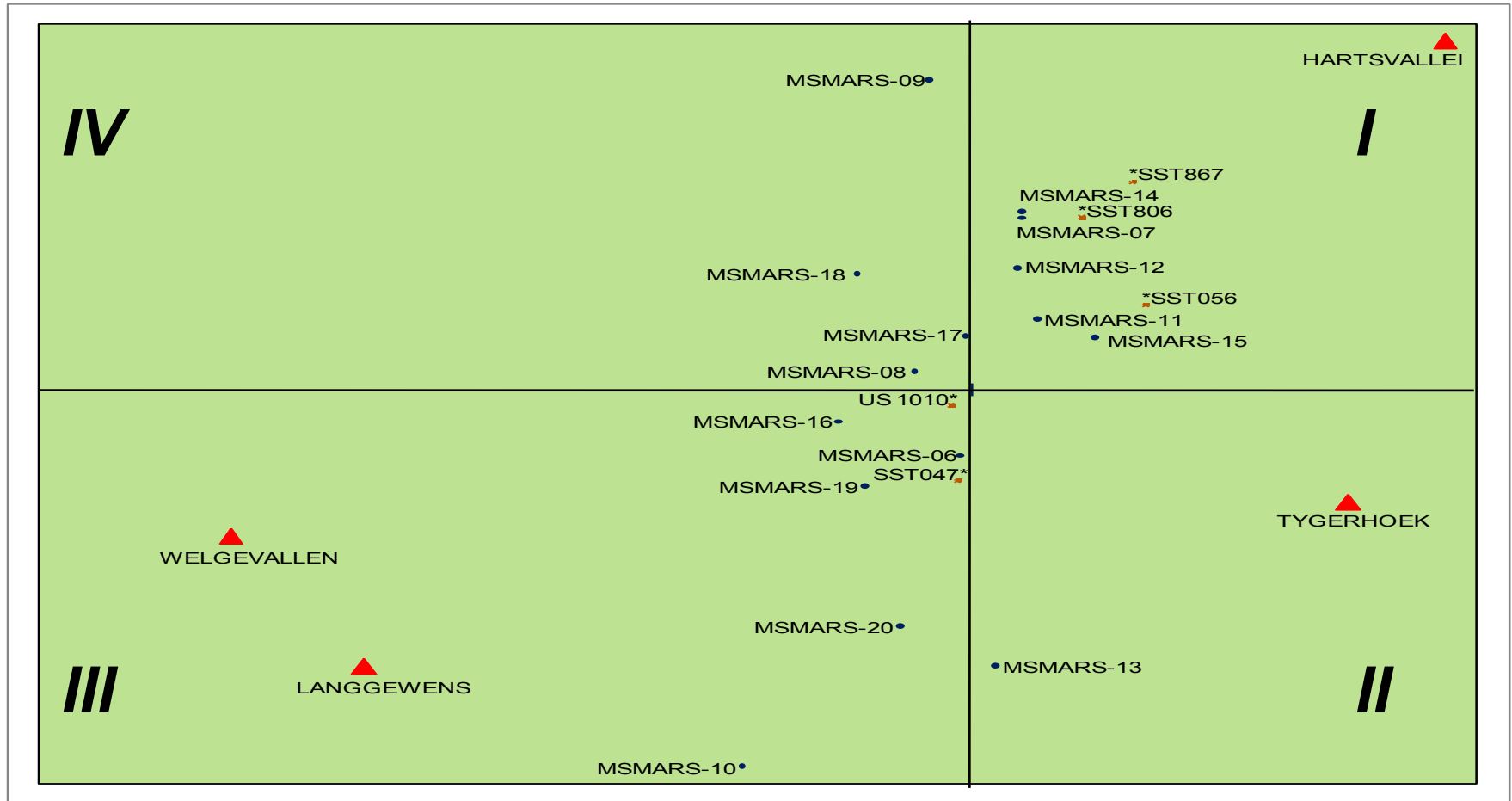
## Case Study/



Name	Deviation from SST806	Quality	Quality deviations
MS-MARS-15	0	Above Average	No deviation from SST806
MS-MARS-19	0	Above Average	No deviation from SST806
MS-MARS-08	1	Average	Problem with VOL
MS-MARS-09	1	Average	-Problem with HLM
MS-MARS-18	1	Average	Problem with EX
MS-MARS-20	1	Average	Problem with HLM
MS-MARS-13	2	Average	Very good EX and PROT. Problem with FL and ABS

# AMMI-Biplot

## Case Study/



# MLFT

## Case Study/



Group	Name	Adaptability and stability characteristic
1	SST056	Very well adapted to low and high potential environments in South Africa
	MS-MARS-11	
	MS-MARS-12	
	MS-MARS-15	
2	SST867	Adapted to low and high potential environments in South Africa
	SST806	
	MS-MARS-07	
	MS-MARS-14	
3	MS-MARS-13	Very well adapted to the dryland high potential environments in South Africa
4	MS-MARS-08	Adapted only to high potential environments in South Africa
	MS-MARS-17	
	MS-MARS-18	
5	MS-MARS-10	Adapted to low potential environments in South Africa
	MS-MARS-20	
6	MS-MARS-09	Adapted only to high potential irrigation environment in South Africa
7	US1010	Stable
8	SST047	Poor performing and not very well adapted or stable
	MS-MARS-06	
	MS-MARS-16	
	MS-MARS-19	

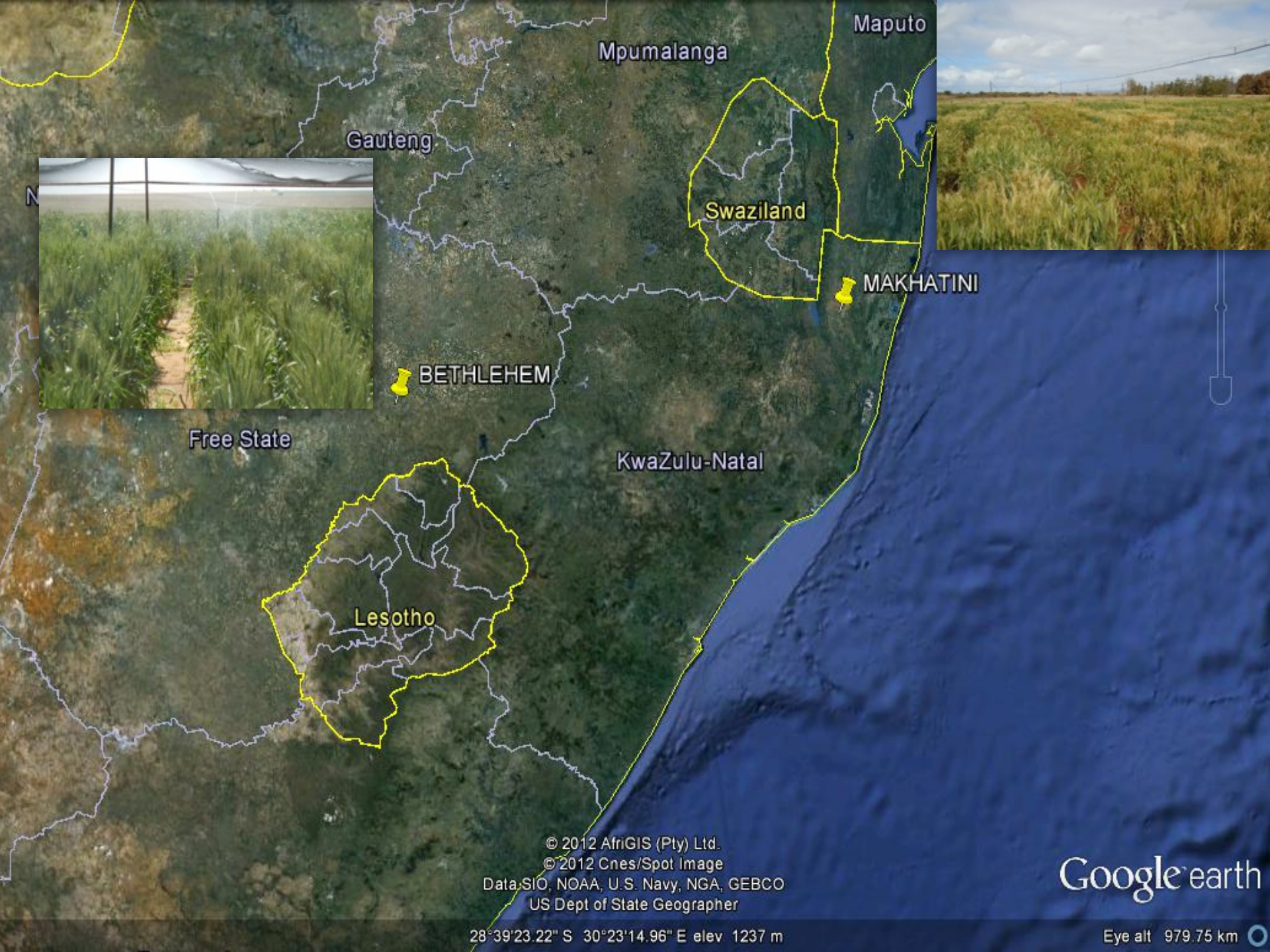
# Field rust screening

Case study/

- Each of the lines were screened for adult plant resistance against the following rust pathotypes:
  - Stem rust – UVPgt60
    - Virulent for *Sr24*, *Sr31* and *Sr38*
  - Leaf rust – UVPrt13
    - Virulent for *Lr24* and *Lr26*
  - Stripe rust – 6E22A-
    - Virulent for *Yr17*







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Data: SIO, NOAA, U.S. Navy, NGA, GEBCO  
US Dept of State Geographer

Google earth

28°39'23.22" S 30°23'14.96" E elev 1237 m

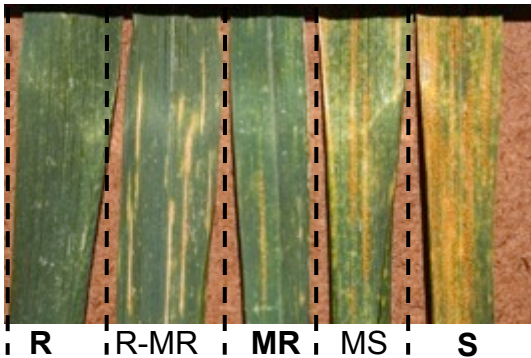
Eye alt 979.75 km



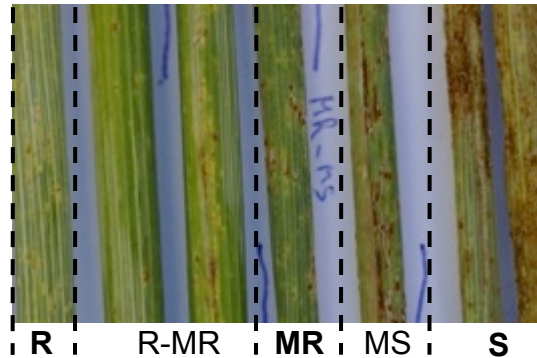
# Field rust screening

Case study/

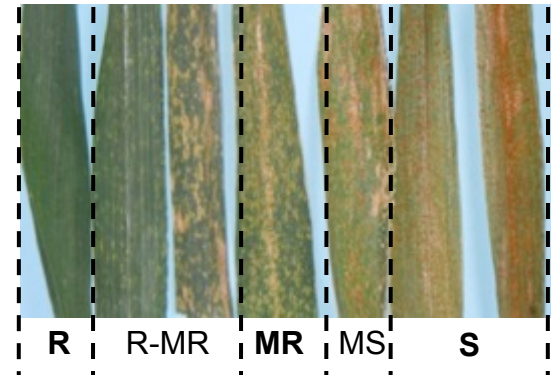
***Stripe rust***



***Stem rust***



***Leaf rust***



# Field rust screening

Case study/



Group	Name	Sr2	Lr34/Yr18	Sr24/Lr24	Sr31/Lr26/Yr9	Sr38/Lr37/Yr17
1	SST 047	-	-		-	
	SST 806	-	-	-	-	-
2	SST 867	-	-	-	-	-
	US 1010	-	-	-	-	-
3	MS-MARS-06	-	-	-		-
4	MS-MARS-08	-				
	MS-MARS-07	-				-
5	MS-MARS-10	-				-
	MS-MARS-14	-				-
	MS-MARS-20	-				-
6	MS-MARS-09	-			-	-
	MS-MARS-17	-			-	-
	SST 056	-			-	
7	MS-MARS-11	-			-	
	MS-MARS-15	-			-	
	MS-MARS-12	-	-			-
8	MS-MARS-13	-	-			-
	MS-MARS-16	-	-			-
9	MS-MARS-18	-	-		-	-
	MS-MARS-19	-	-		-	-

# Conclusion

Case study/



Evaluation	Specific characteristic	MS-MARS lines
<i>General yield adaptability and stability</i>	Low and high potential	MS-MARS-07, 11, 12, 14 & 15
	Low potential	MS-MARS-13
<i>Bread quality</i>	Above average	MS-MARS-15 & 19
	Average	MS-MARS-08, 09, 13, 18 & 20
<i>Rust resistance</i>	Minor genes for the three rust pathogens	MS-MARS-15 & 18
	Minor genes for two rust pathogens	MS-MARS-07 & 09



# Conclusions

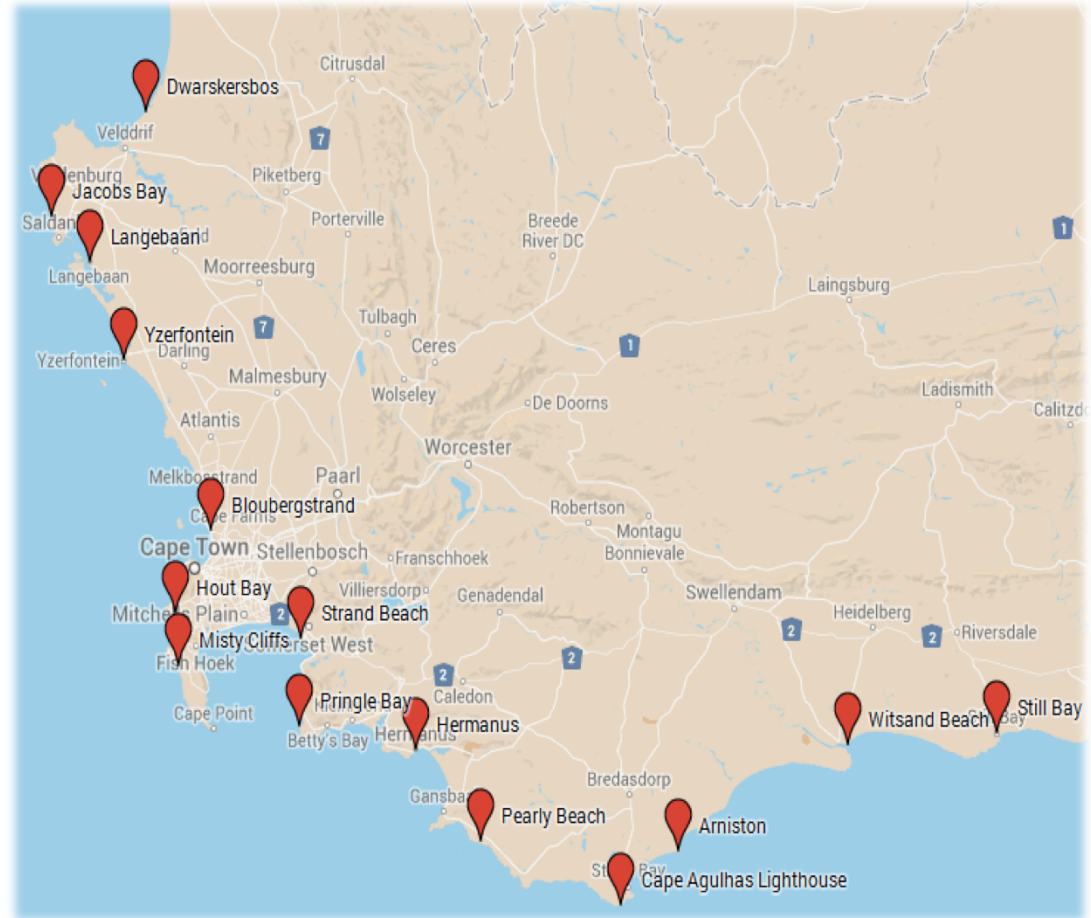
Case study/



- Breeding strategy relies on:
  - Mode of reproduction
  - Traits to be improved
  - Time available
  - Resources
- MS-MARS scheme applied to most crops and traits regardless of the mode of reproduction or nature of the traits (qualitative and quantitative) to be improved
- DHs assist in shortening the generations needed for obtaining pure breeding material (one vs. six generations).
- Requires costly resources in the form of highly skilled labour and equipment
- Innovative approach to combine MAS and a biotechnology tool such as DHs

# Salinity tolerance

Current activities/







# Physiological traits

Future activities/

- Expanding current MS-MARS based pre-breeding activities to national platform
- Funded by GrainSA, DST and industry
- All of the SA wheat breeding programs involved
- Primary aim is improvement of yield
- Physiological traits of primary interest
- UAV based high throughput phenotyping to be implemented
- Move towards phenomics approach







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