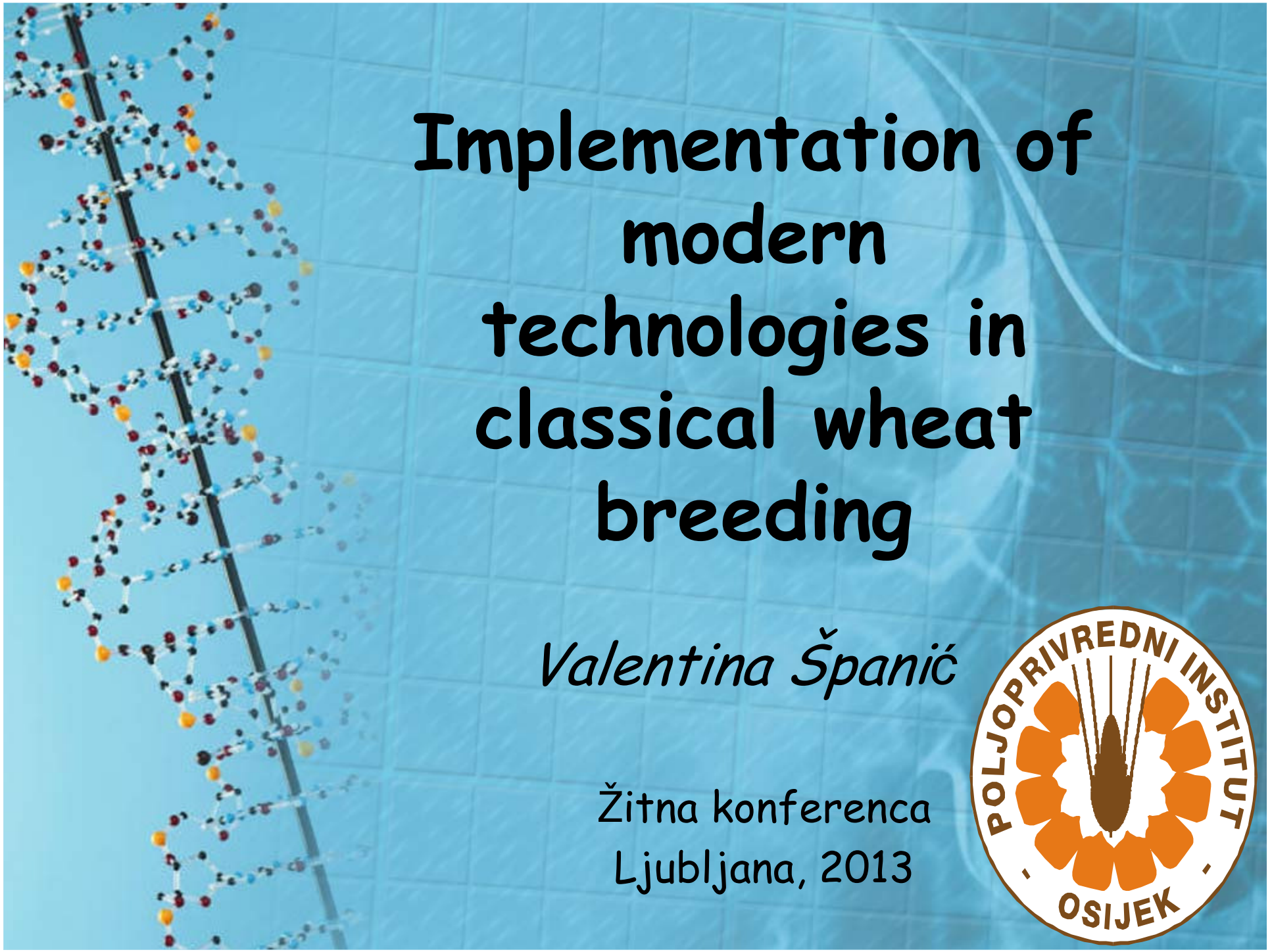
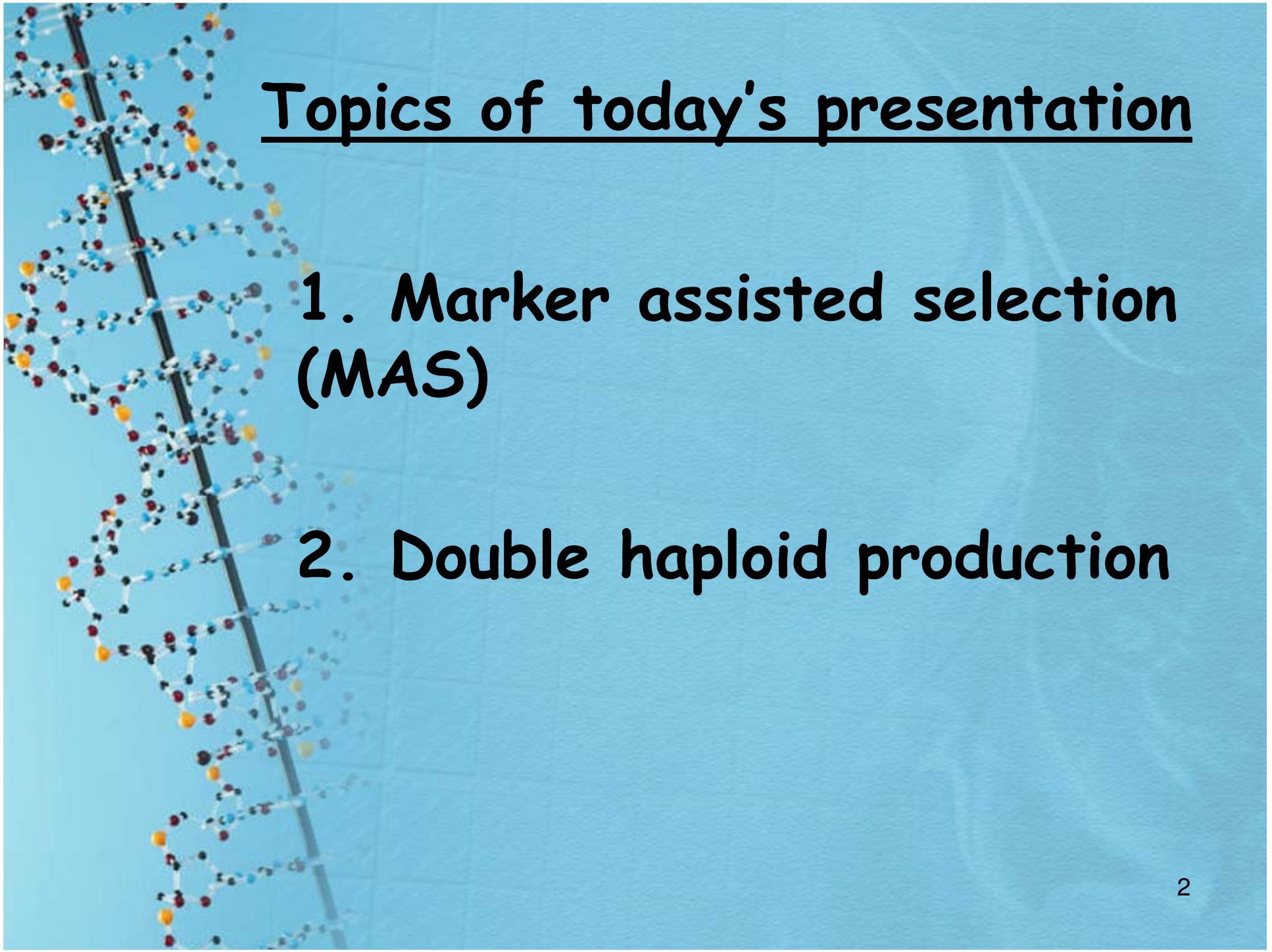


Implementation of modern technologies in classical wheat breeding

Valentina Španić

Žitna konferenca
Ljubljana, 2013

POLJOPRIVREDNI INSTITUT OSIJEK



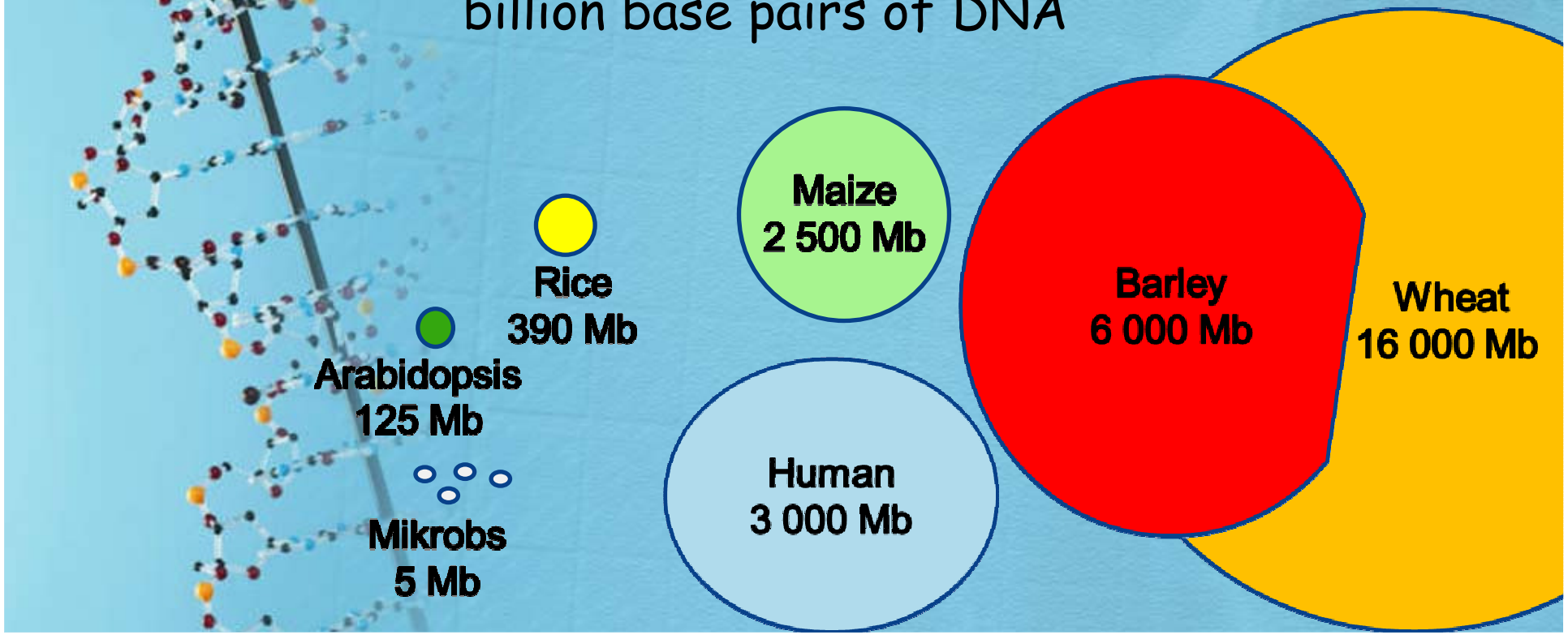
Topics of today's presentation

1. Marker assisted selection (MAS)

2. Double haploid production

1.Marker assisted selection

- Common wheat (*Triticum aestivum* L.) has one of the largest and most complex genomes of cereals (2n = 6x = 42, AABBDD)
- Total size of haploid wheat genome is 16 billion base pairs of DNA



Genetic Markers

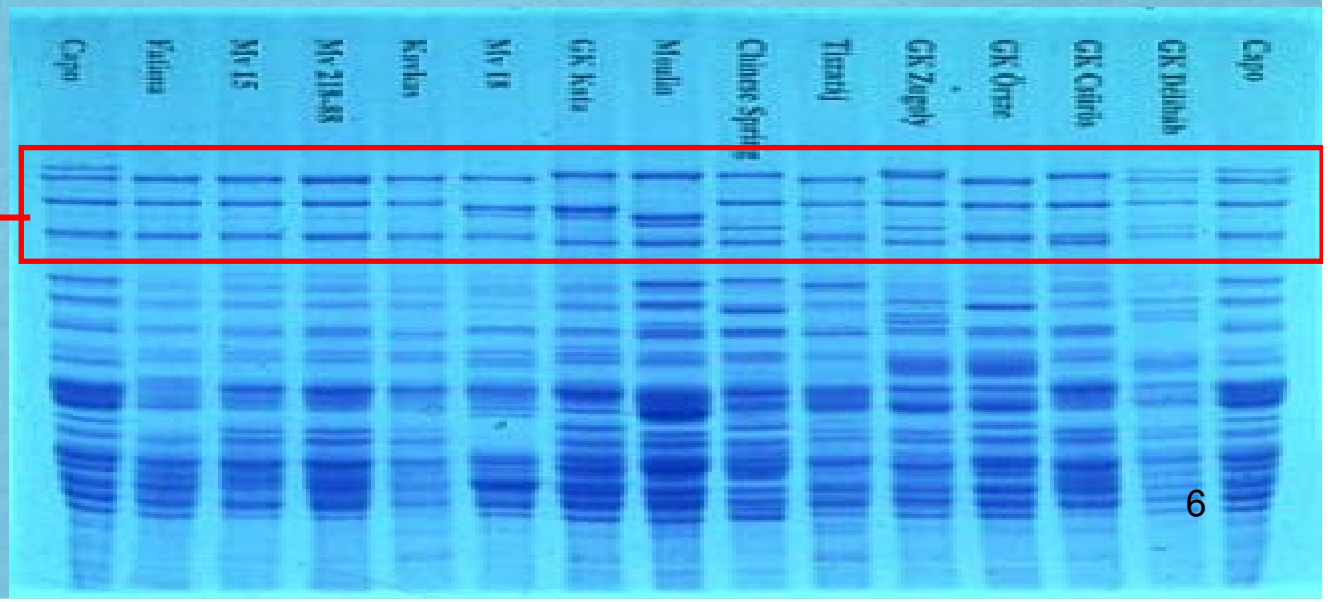
- genetic diversity among organisms or species
- serve as an indication for specific genes and are located near or associated with the genes of interest

Three types of genetic markers:

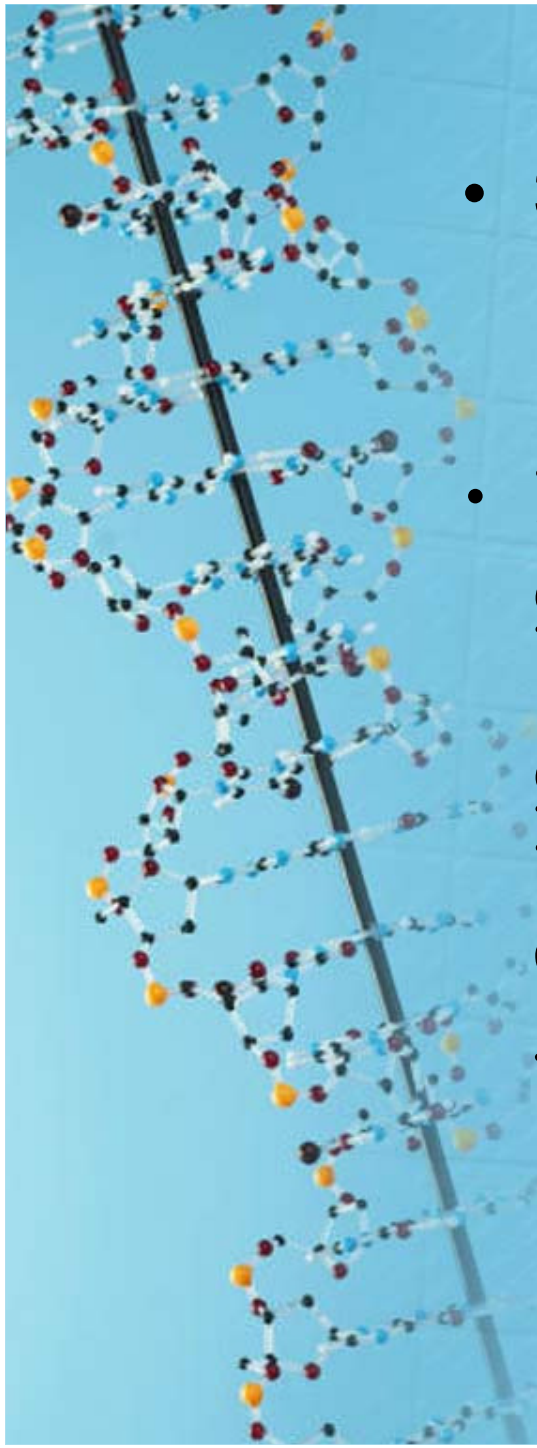
- **1. Morphological markers**
- (classical or visible) markers, which are, in fact, phenotypic properties.

owns

- **2. Biochemical markers**
- include allelic variants of enzymes called isoenzymes. The main disadvantage of morphological and biochemical markers is that they may be limited in number and are influenced by environmental conditions or developmental stages of plants



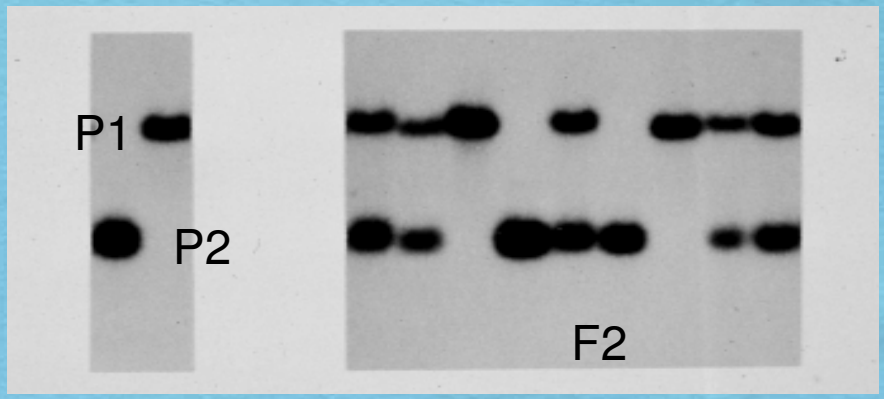
High molecular weight glutenins

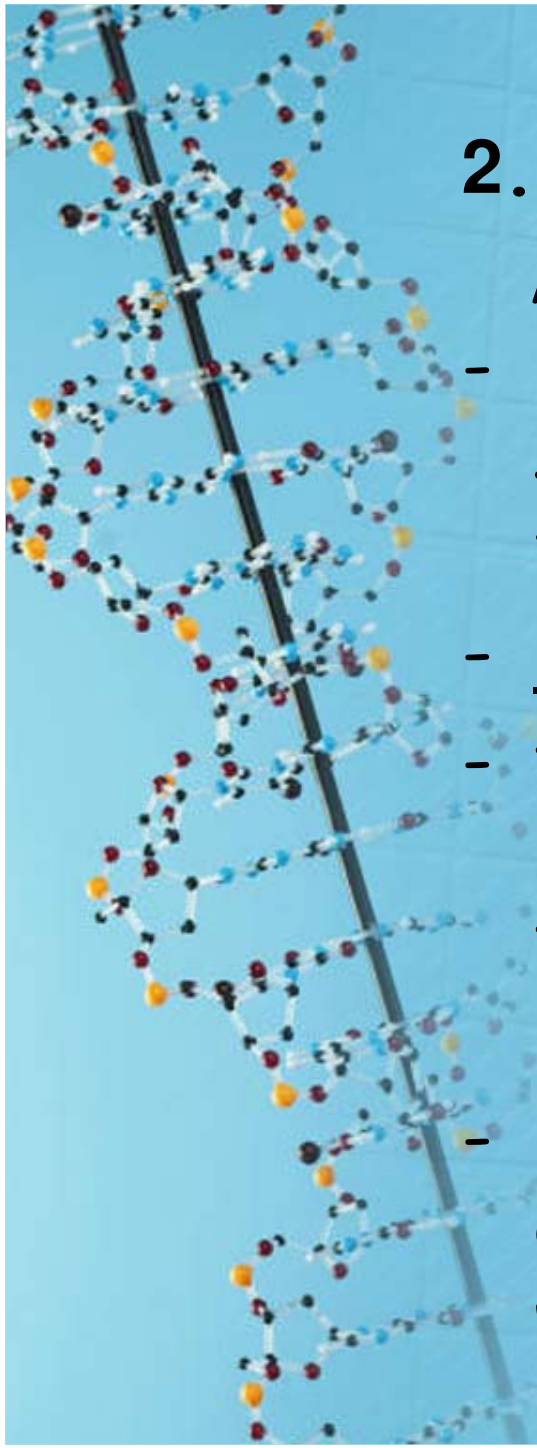


- **3. DNA (molecular) markers**, which reveal the sites of variation in DNA.

- It is possible to determine the level of genetic diversity within germplasm, to identify genotypes and to estimate the genetic distance between populations, to detect QTL's, to develop marker-assisted selection (MAS), to identify sequences of useful genes.

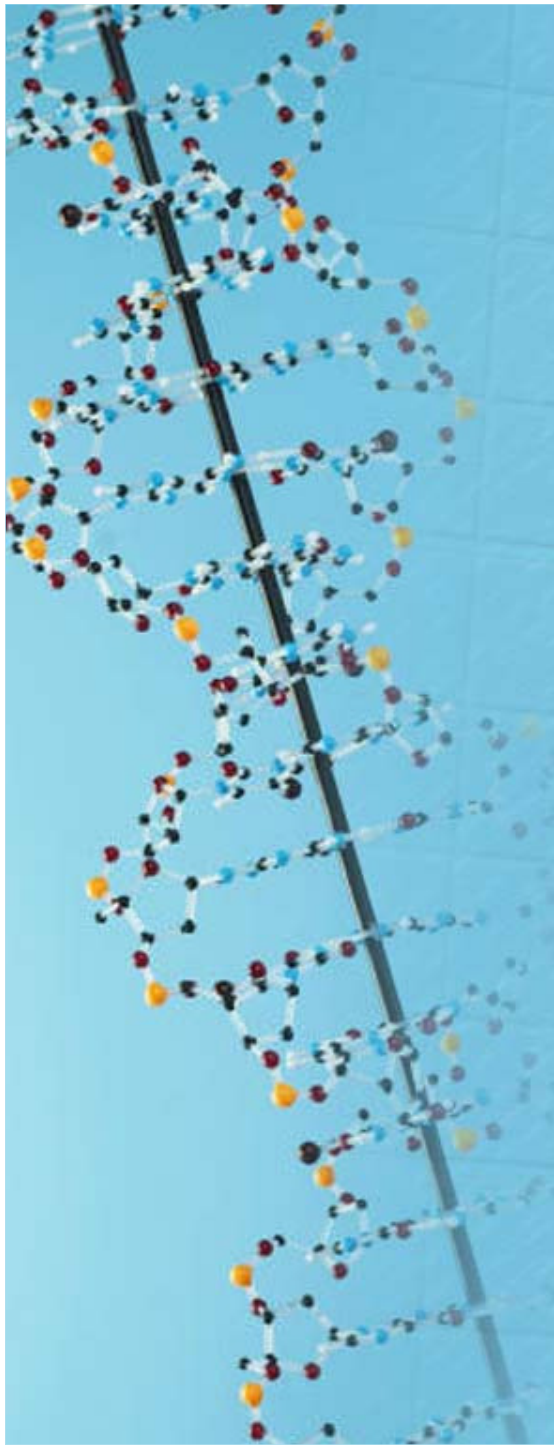
- DNA markers used in the construction of molecular maps of wheat are classified into few groups:
- **1. RFLP** (*restriction fragment length polymorphisms*)
- detect small differences between the two DNA fragments on the basis of presence or absence of a restriction site





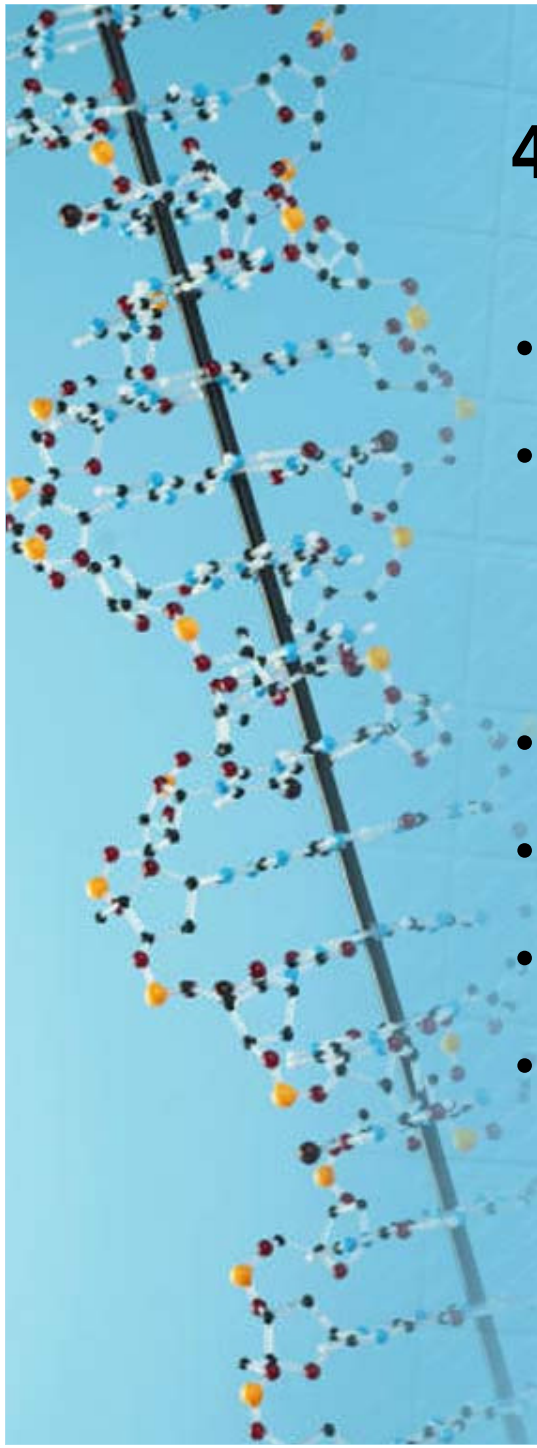
2. **RAPDs** (*randomly amplified polymorphic DNAs*);

- requires the presence of a randomly selected oligonucleotide that acts as a foward and reverse primer
- <u>Disadvantages</u>
- the response for the same type of plant may not be reproducible due to the existing variations in different laboratories
- may remain a question of the presence of RAPD bands of similar molecular weight but different sequence.



3. AFLPs (amplified fragment length polymorphisms)

- they are based on the principles of selective amplified restriction fragment from a complex mixture of DNA obtained after digestion of genomic DNA with restriction endonucleases
- dominant markers
- High reproducibility, rapid generation, covering the whole genome, high polymorphism

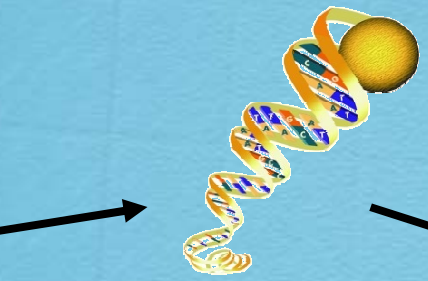


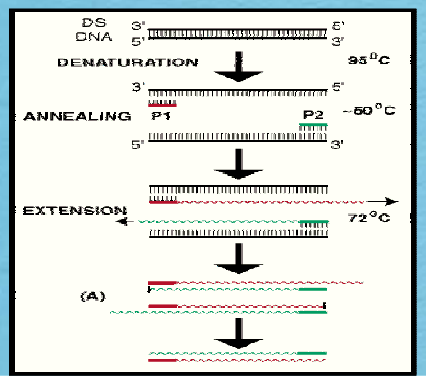
4. **SSRs** (*simple sequence repeats* or microsatelites)

- a motif length of 2 to 5 nucleotides
- sequences seem to be present evenly distributed all over the plant genome, frequently found within genes
- co-dominant markers
- the handling of microsatellites is easy
- several alleles can be expected
- they ensure a high level of polymorphism in hexaploid wheat

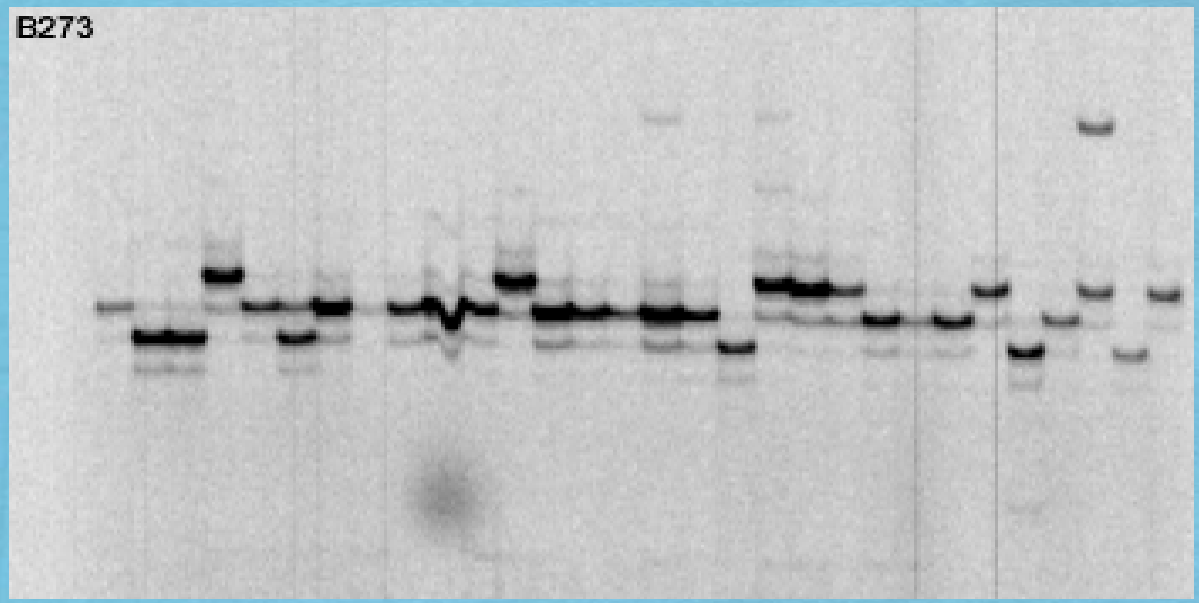
Molecular analysis of genetic diversity

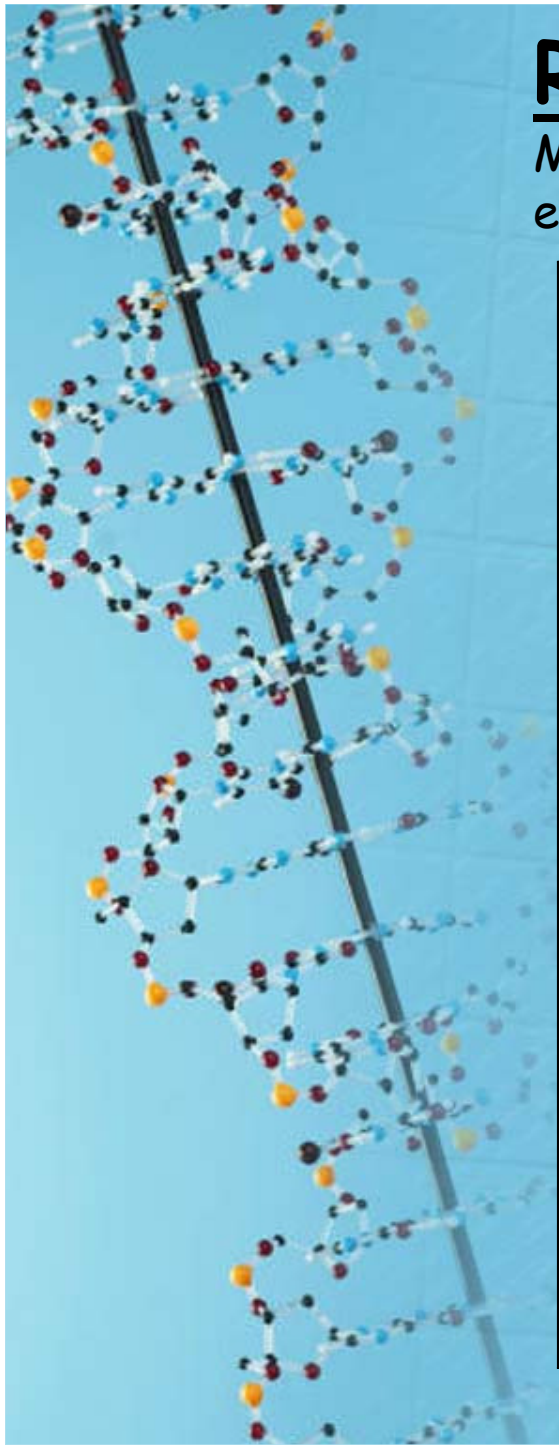
DNA extraction was performed according to the modified CTAB-method described by Saghai-Maroof et al. (1984) (CTAB extraction bufer, kloroform-izoamil (24:1) alcohole, izopropanol, wash II, TE-bufer, concentration of DNA 100 ng/µl)





For directly labeled primers were used different PCR programs, depending on the temperature sticking primers, and for M-13 primers were used touch down (gradually lowering the temperature) programs Microsatellite analyzes were performed using fluorescent detection of fragments in the LI-COR 4200 DNA sequencing system (0.7% polyacrylamide gel)

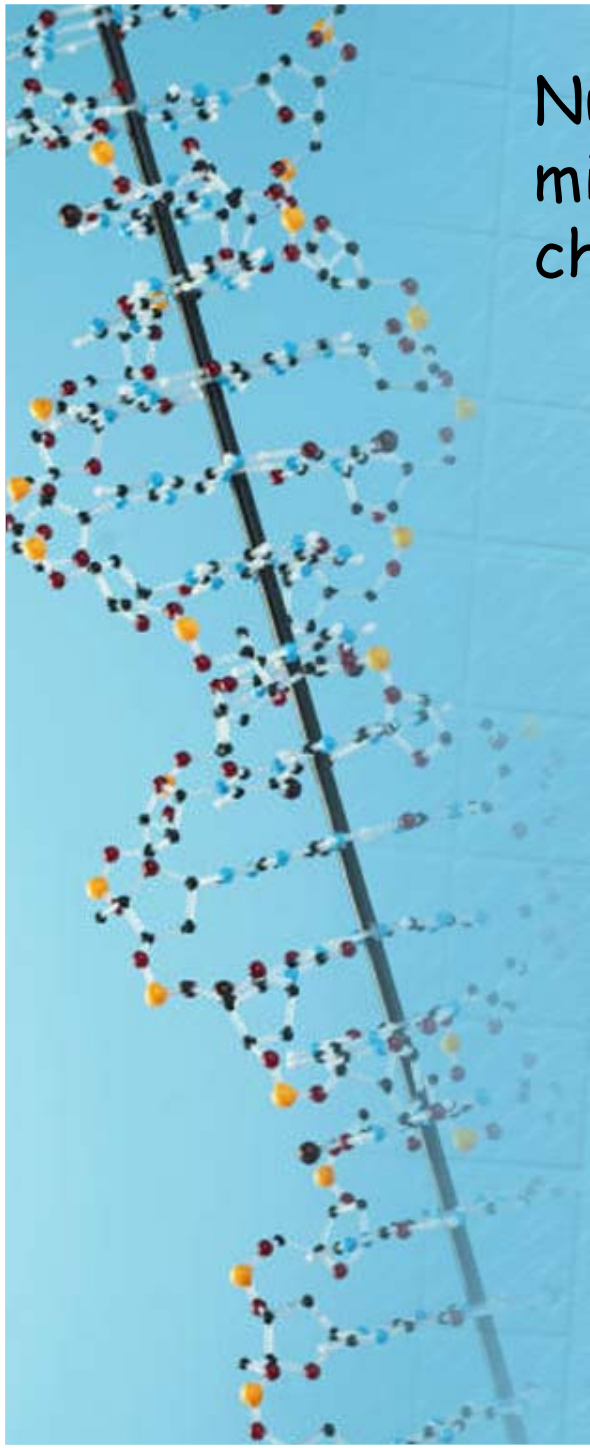




Results

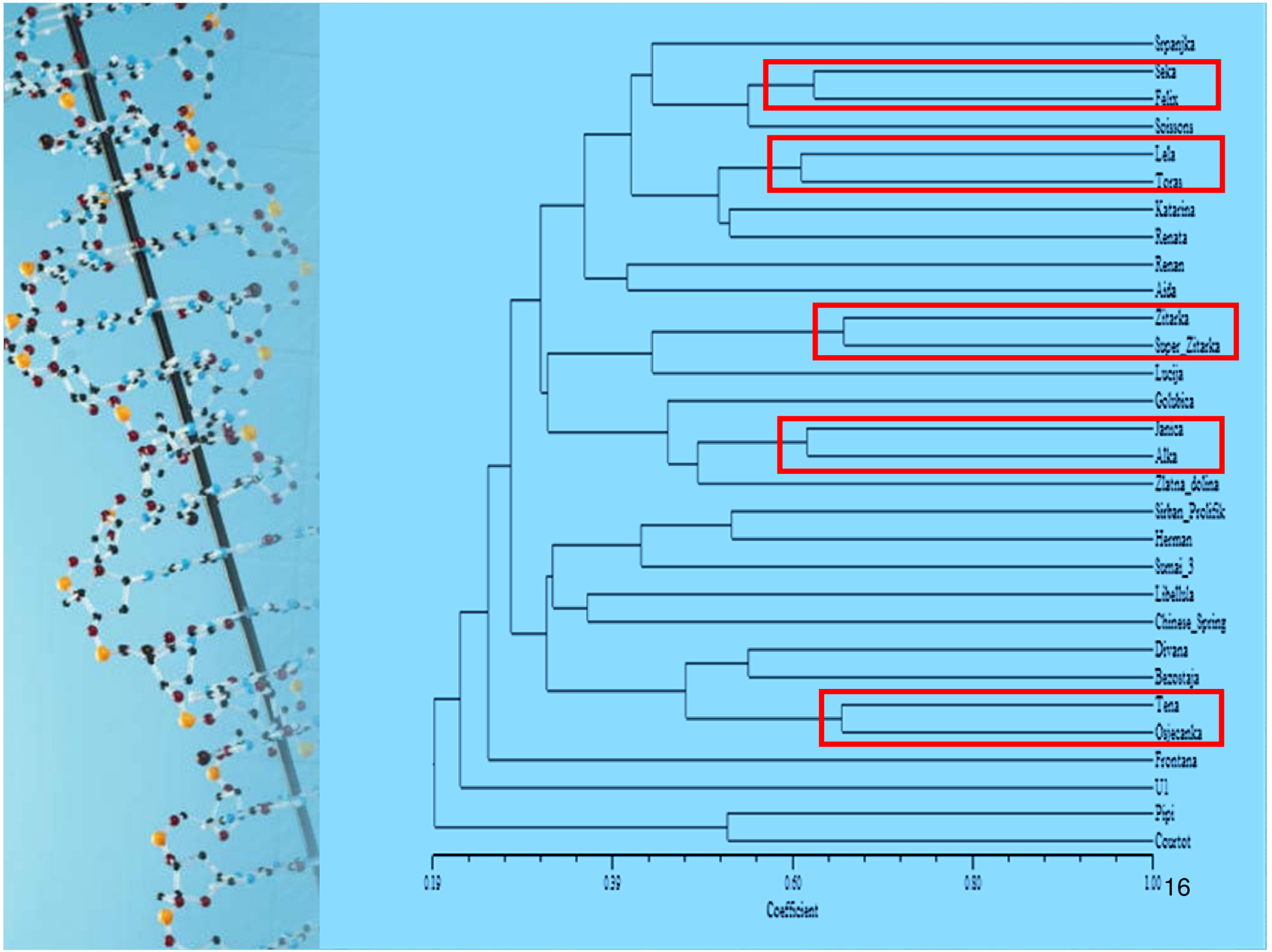
Microsatellite markers, their chromosomal location, the expected allele size, number of alleles and PIC value

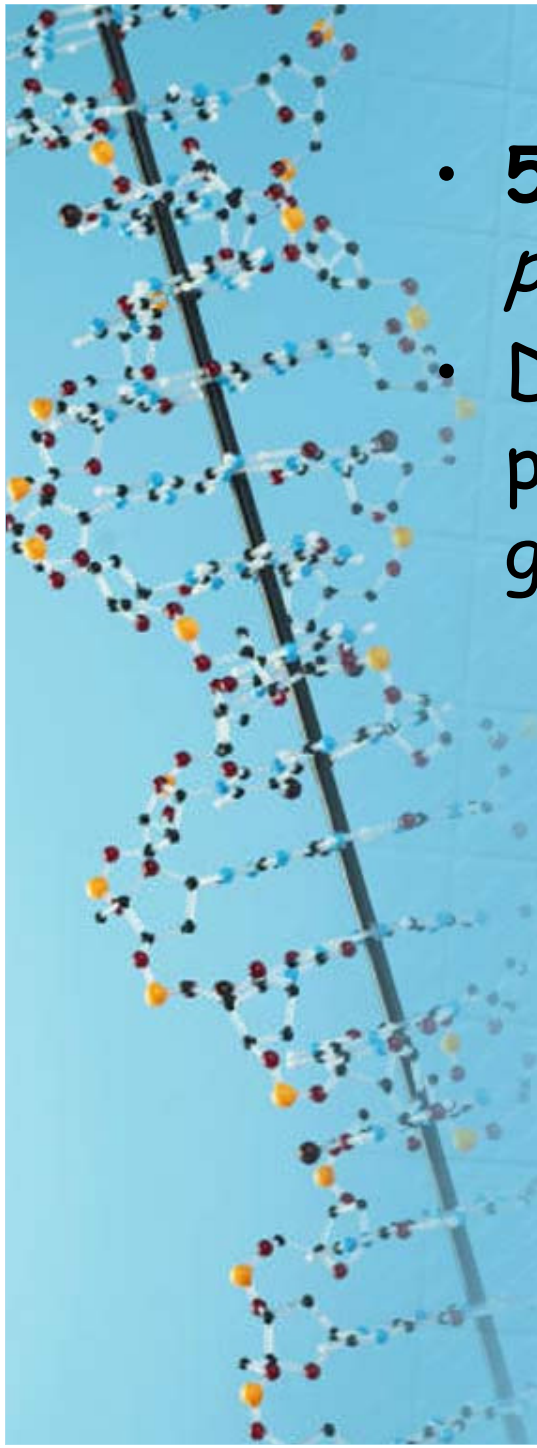
Mikrosatellite marker	Chromosomal location	Expected size of alleles (bp)	Number of alleles (N_a)	PIC
Xgwm164	1A	120	7	0,76
Xgwm642	1D	180-200	5	0,69
Xgwm558	2A	-	7	0,75
Wmc667	2A	-	10	0,82
Xgwm120	2B	150-170	9	0,82
Xgwm349	2D	210-260	6	0,76
Xgwm1071	3A	150	8	0,76
Barc84	3B	-	2	0,07
Xgwm160	4A	180	5	0,56
Xgwm610	4A	170	6	0,81
Xgwm888	4B	195	1	0,08
Xgwm624	4D	130-140	9	0,84
Barc56	5A	125	6	0,66
Barc319	5A	-	7	0,82
Xgwm408	5B	-	7	0,67
Xgwm335	5B	200-240	9	0,82
Xgwm190	5D	200-250	2	0,24
Barc3	6A	-	5	0,75
Xgwm427	6A	180-200	6	0,79
Xgwm219	6B	150-190	8	0,83
Xgwm816	6B	180-190	5	0,72
Barc273	6D	225-240	3	0,60
Xgwm681	7A	190	14	0,90
Xgwm870	7A	135	5	0,78



Number of alleles and the number of microsatellites used for genome chromosomes

Genome	Number of alleles	Number of microsatellites
A	7,17	12
B	5,86	7
D	5,00	5
Chromosome		
1	6,50	2
2	8,00	4
3	5,00	2
4	5,25	4
5	6,20	5
6	5,40	5
7	9,50	2


Španjska
Seka
Felix
Soissons
Lela
Toras
Katarina
Renata
Renan
Aida
Zitarka
Super_Zitarka
Lucija
Golubica
Janica
Alka
Zlatna_dolina
Srbian_Prolific
Herman
Somai_3
Libellula
Chinese_Spring
Divana
Bezostaja
Tena
Osjecanka
Frontana
U1
Pipi
Cooltot
0.19
0.39
0.60
0.80
1.00
Coefficient



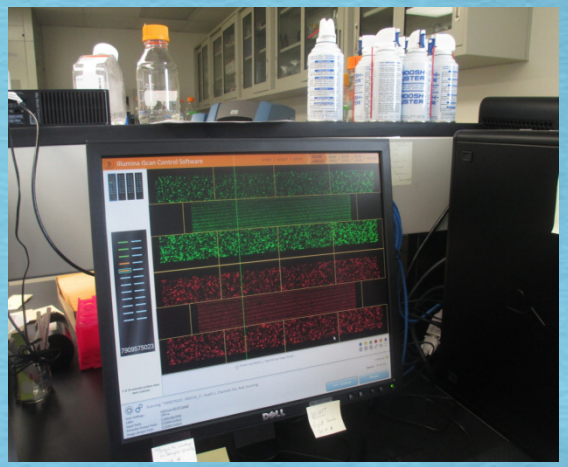
- **5. SNPs** (*single nucleotide polymorphisms*)
- DNA variation, which occurs when a particular nucleotide A, T, C or G in the genome varies in different species

InDels (*insertion-deletions*)

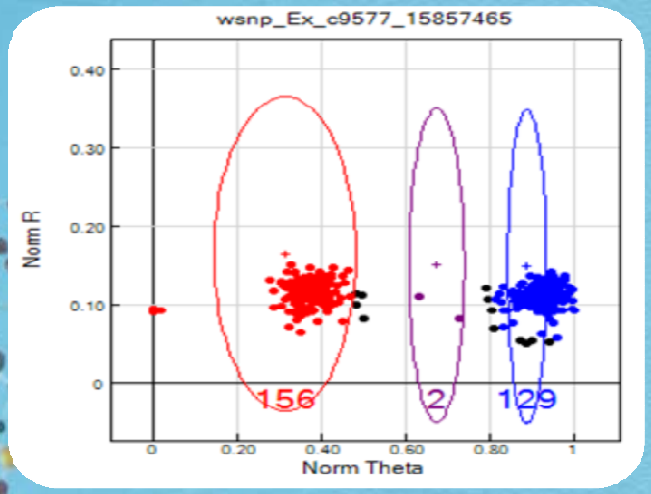
Small insertions and deletions

QTL mapping

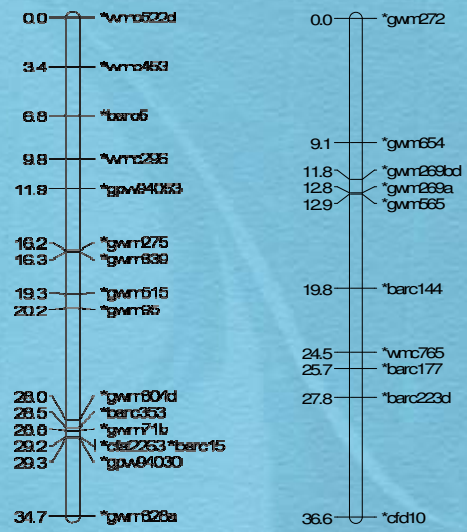
DNA isolation, Biosprint



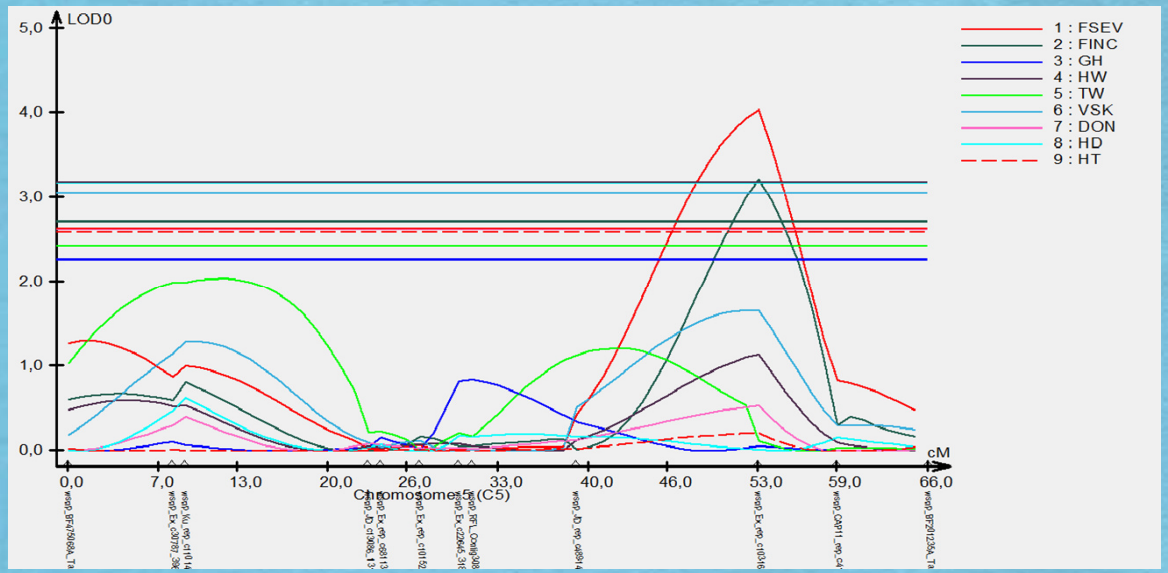
Genotyping, 9 000 SNP markers



Polimorphism, Illumina Genome studio



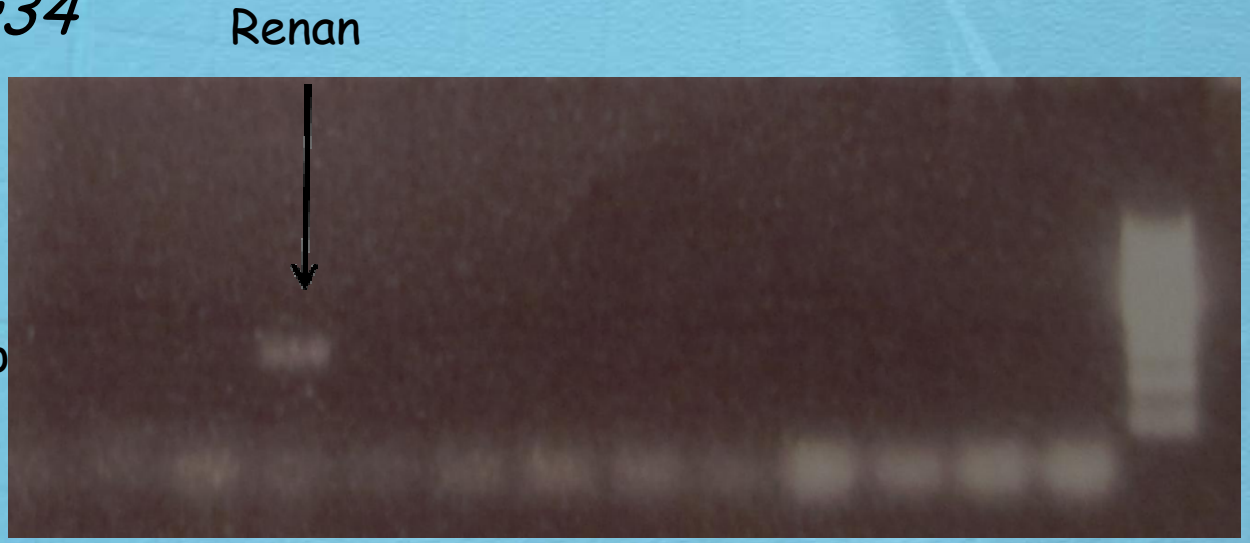
Maps (JoinMap program (version 4.1 for the PC)



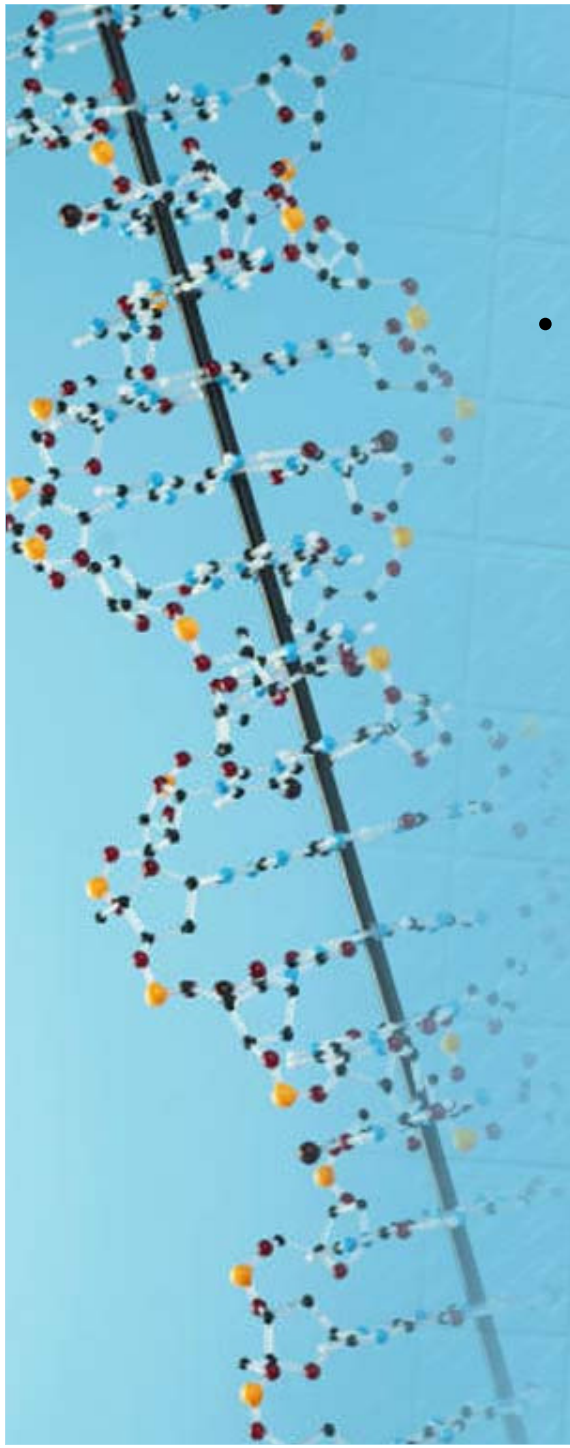
QTLs (QTLCartographer)

Diagnostic molecular markers

- *Lr34*



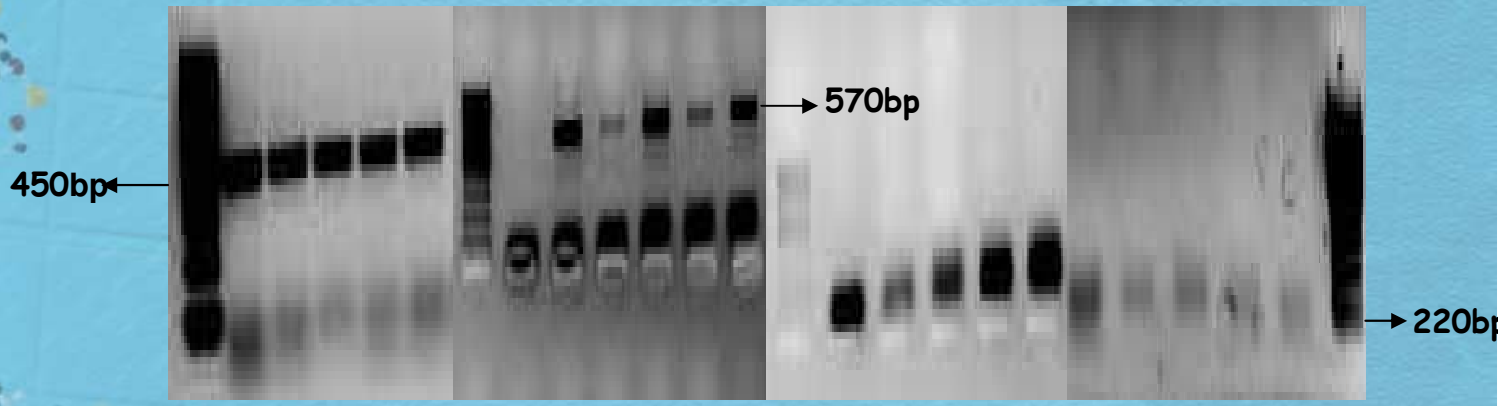
Leaf rust: *Lr13, Lr21, Lr24, Lr34 and Lr37*
PinA and PinB (hardness), Waxy (starch properties), BYDV and BYAgi (BYD virus resistance), GPC (grain protein quality), Glu-A1/GluD1/Glu-D2 (HMW glutenin)
Fhb13BS and *Fhb5A* (Fusarium head blight)



Lr34/Yr17/Sr38

- *Lr34* gene was mapped to the short arm of chromosome 7D. This gene is also linked to leaf tip necrosis. Cultivars with *Lr34* should also have adult-plant resistance to stripe rust (*Yr18*). This locus also exhibits a pleiotropic effect on barley dwarf virus reaction (*Bdv1*) described as „slow yellowing" response in adult plants. Also this gene often can interact with seedling resistance genes in seedling plants to produce lower than expected infection types.

Species-specific primers



- Confirmation of analyzed strains as *F. graminearum* (450-bp), *F. culmorum* (570-bp), *F. avenaceum* (220-bp) and *F. poae* (220-bp) from winter wheat kernels in East Croatia.

2. Wheat breeding -aided with double haploid production

- Traditional (classical) breeding process takes many years and breeders need to create a large number of plants under certain conditions
- Creating cultivars - can take up to 14 years
- It is necessary to bring together traditional and modern breeding
- Double haploids could shorten this process, because homozygosity can be achieved very quickly

- Haploids - plants which have in their sporophit gametofit number of chromosomes
- They occur in many plant species, either as a result of parthenogenesis (when unfertilized egg, sperm or sinergida begin to grow and create an independent haploid plant), either as a result of experimental techniques
- Usage: in the determination of mutation and detection of unique recombinate and doubling their number of chromosomes obtained homozygous cells (the clean lines of wheat)

Haploid may be experimentally obtained by stimulating the egg, sinergids and spermal cells to cell division with number of methods:

- ionizing radiation,
- radioisotopes,
- temperature shock,
- distant hybridization,
- late pollination,
- application of dysfunctional pollen,
- protoplast culture,
- elimination of chromosomes in the culture of young embryos,
- parthenogenesis in vitro

WHEAT DOUBLE HAPLOIDS

- In classical breeding chances to get a completely homozygous lines are rare, and most of the genotypes have heterozygous locus, which significantly reduces the accuracy of selection
- Double haploids should be genetically stable
- Wheat double haploids can be produced through anther culture of wheat or intergenetic crossing with barley, maize or various herbs belonging to the family *Gramineae*

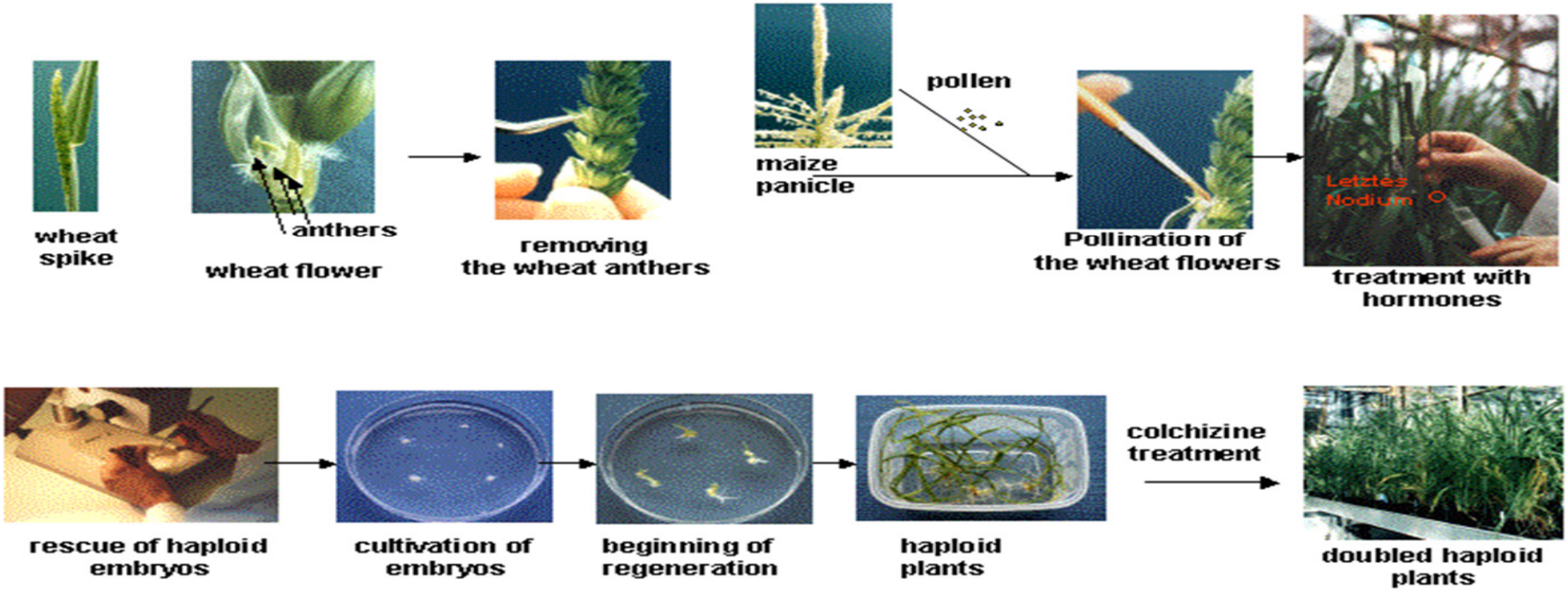
- Intergenetic crosses between wheat and maize, followed by elimination of the maize genome and are considered as an effective method for the induction of haploid zigotic embryos
- Haploids obtained by crossing wheat and corn are less dependent on the genotype, and such a technique is simpler and more efficient than crossing wheat with Hordeum bulbosum
- Bulbosum technique, developed from haploid production of barley has been extended to wheat (Barclay, 1975).
- Effect of gene Kr1 and Kr2 in wheat has banned the usage of bulbosum technique for polihaploid production in wheat (Snape et al., 1979).

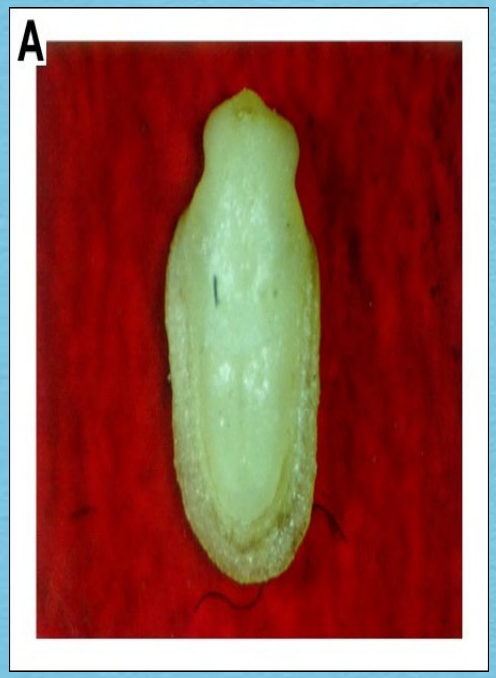
- Maize chromosomes have poorly expressed centromeres and appears to have a little affinity for the spindly mikrotubulus in the zygote and young embryo

- They are lost in the first two cycles of cell division to produce embryos whose cells contain a haploid complement of wheat chromosomes

- Fertilization of wheat with maize is relatively insensitive to the action of dominant alleles of Kr locus

Wheat x Maize - System



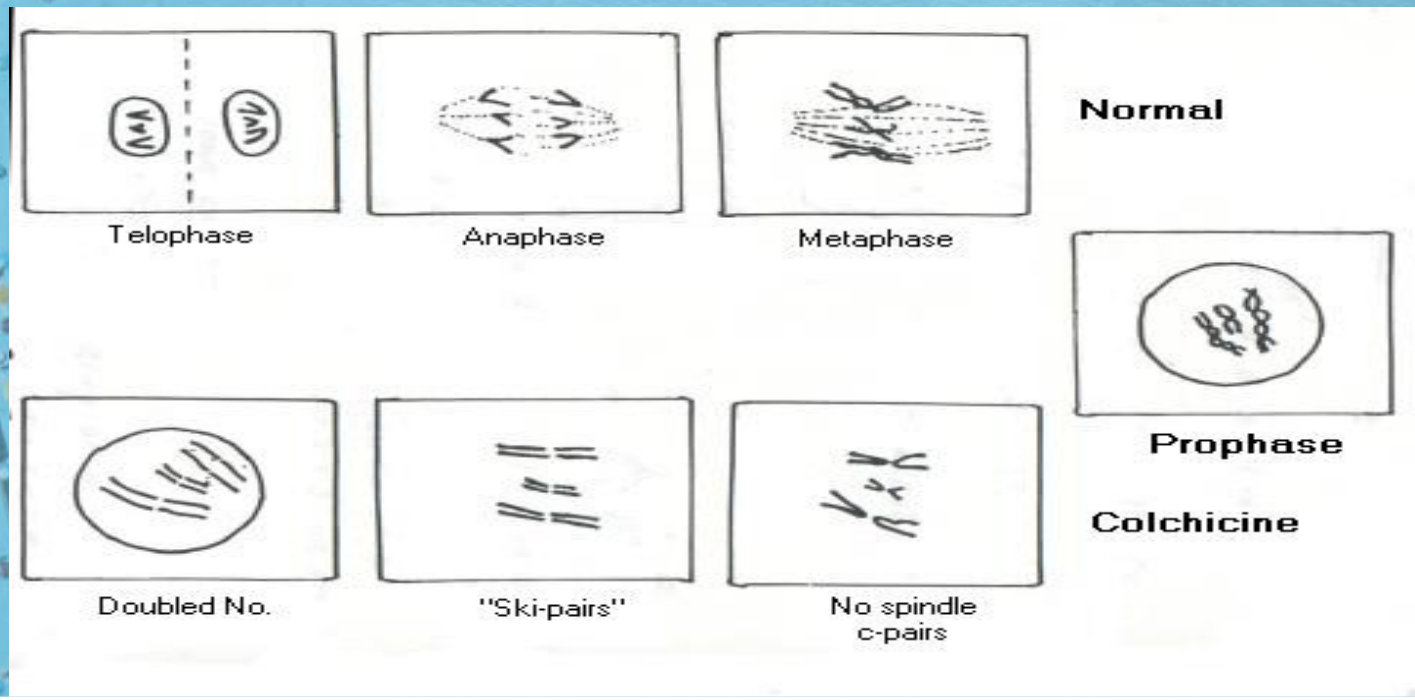


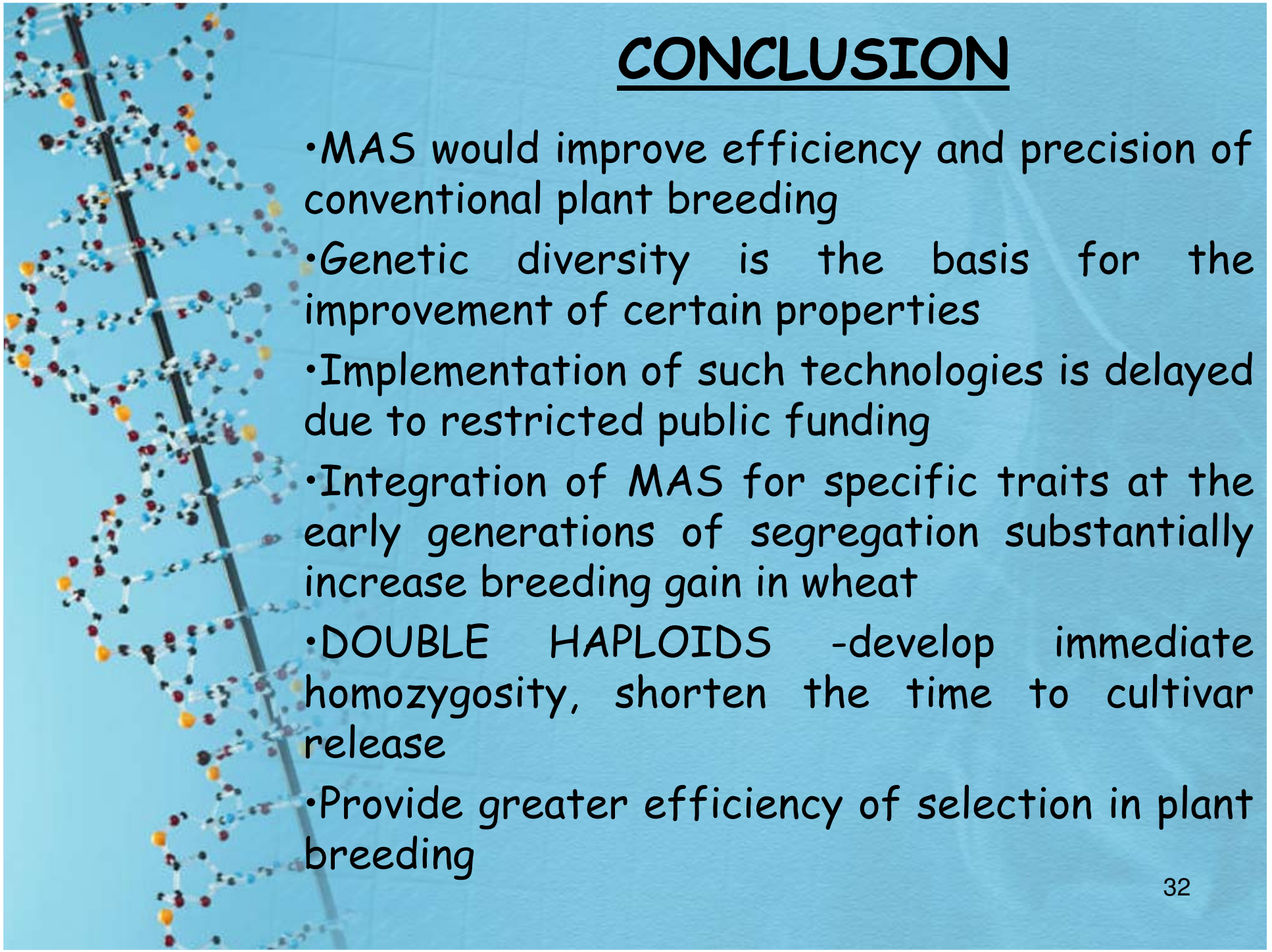
The embryos of 1.5-2 mm are placed on MS medium (Murashige and Skoog, 1962). With addition of 0.5 mg/l nicotinic acid, 0.1 mg/l thiamine HCl, 0.5 mg/l pyridoxine HCl, 2 mg/l glycine, 30 g/l glucose and 7 g/l agar.

PROCEDURE WITH COLHICINE

It is used for inhibition of spindle which allows doubling the number of chromosomes and the creation of poliploid plants

Plantlets in the antero are processed with 0.5% solution of colchicine 24 to 48 hours, washed in sterile water and then transplant the basic media





CONCLUSION

- MAS would improve efficiency and precision of conventional plant breeding
- Genetic diversity is the basis for the improvement of certain properties
- Implementation of such technologies is delayed due to restricted public funding
- Integration of MAS for specific traits at the early generations of segregation substantially increase breeding gain in wheat
- DOUBLE HAPLOIDS -develop immediate homozygosity, shorten the time to cultivar release
- Provide greater efficiency of selection in plant breeding

Thank you for your attention!