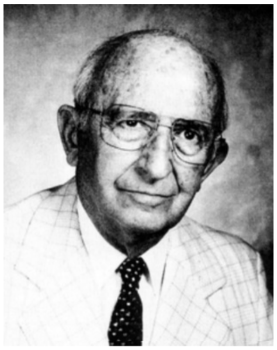
Aneuploids in wheat

Sears, 1954

Review by Gupta & Vasistha, 2018

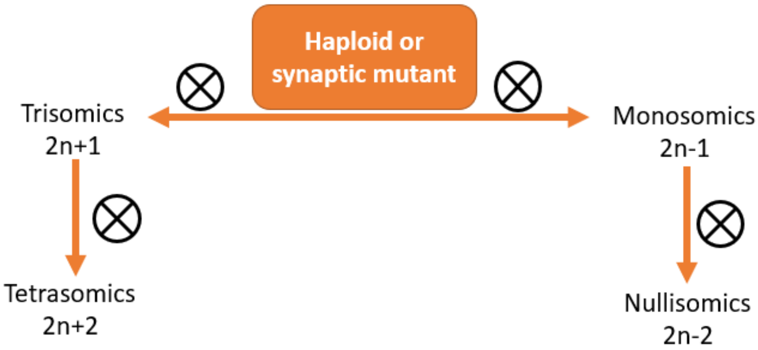


Ernest Robert Sears
(1910-1991)

Wheat × rye → 3 haploids

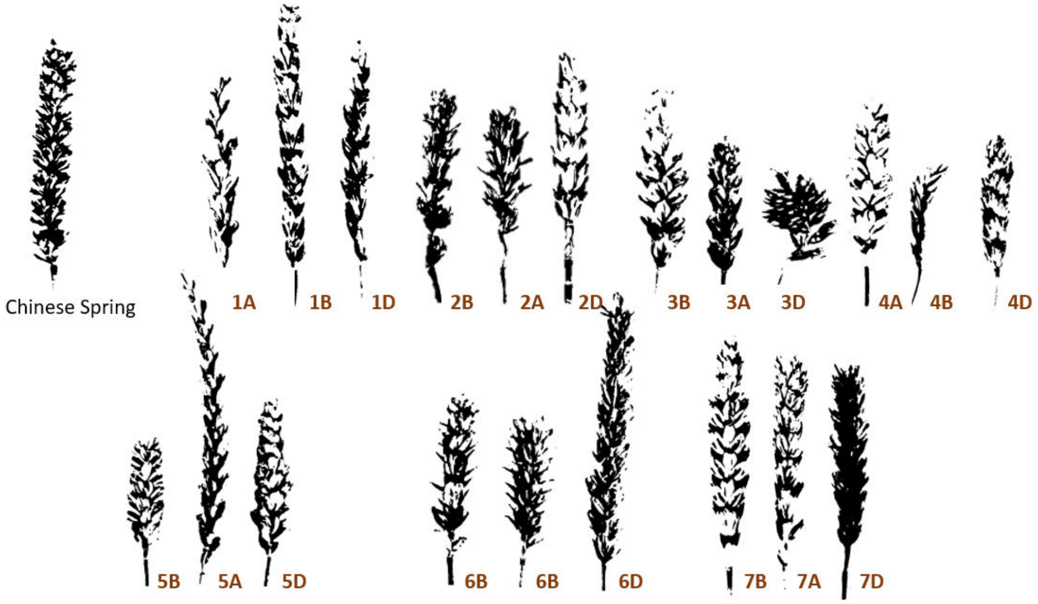
- Used to get an aneuploid series

There are 4 aneuploids available for each chromosome



- Monosomics: hard to tell from normal when grown under good conditions, except for 5A, which is "het speltoid" phenotype
- Trisomics: difficult to tell from normal, except for 5A, which is of the "het compactoid" phenotype
- Tetrasomics: closer to normal than nullisomics. Both male and female fertile, with some reduced fertility.
- Nullisomics: Can identify by seedling or reproductive traits. Are partially fertile as male or female.

Following are the various nullisomic phenotypes of 'Chinese Spring'



Behavior of univalents

- Can separate at 1st division, segregate at random in the 2nd division
- Can remain off the plate at first division, divide during the second
- Mis-divide, forming either isochromosomes or telosomes
- Can be lost altogether

Behavior of monosomes

n-1 ♀\♂	n (96%)	n-1 (4%)
n (25%)	2n (24%)	2n-1 (1%)
n-1 (75%)	2n-1 (72%)	2n-2 (3%)

Their transmission is usually higher through the female than the male

- 2n-1 × 2n → 75% 2n - 1 (♀ produces 25% n eggs and 75% n-1 eggs)

- 2n × 2n-1 → 3% 2n-1 (produces 90-98% n sperm, and 2-10% n-1 sperm)

Thus, a monosomic can be maintained by ⊗, which results in 70-75% monosomics

⊗ 2n-2 → >90% 2n

Behavior of trisomes

2n+1 × 2n → 40% 2n + 1 (produces 60% n eggs and 40% n+1 eggs)

n+1 ♀\♂	n (75-80%)	n+1 (2-25%)
n (60%)	2n	2n+1
n+1 (40%)	2n+1	2n+2 (1-10%)

2n × 2n+1 → 7% 2n+1 (♂ produces 75-98% n sperm, and 2-25% n+1 sperm)

Thus, a trisomic can be maintained by ⊗, which results in 31-54% trisomics

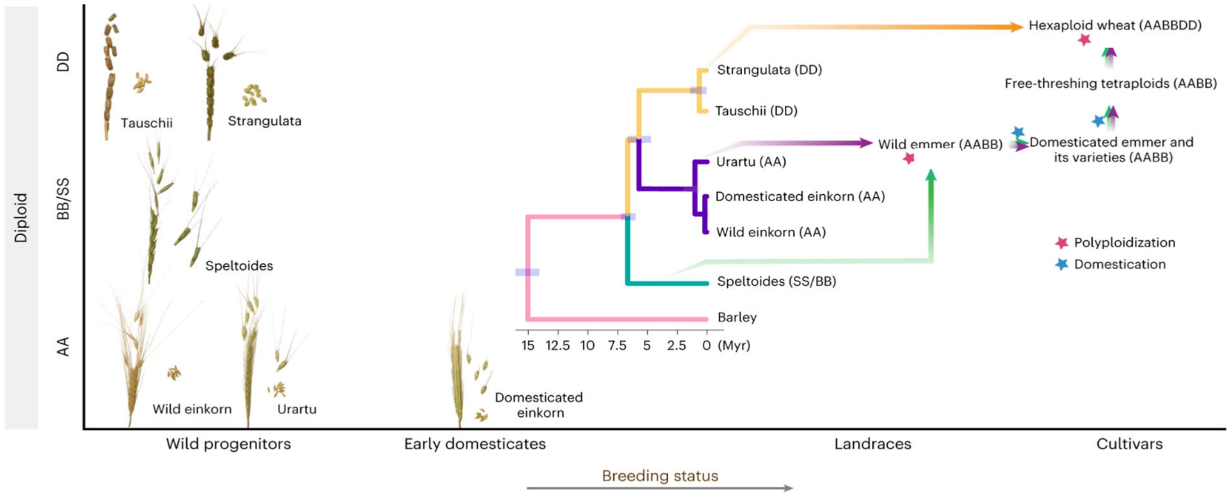
⊗ 2n+2 → 80+% 2n+2

Transmission rate of small chromosomes is greater than that of larger chromosomes

The wheat genome

Formation

Zhao et al, 2023



Assigning individual chromosomes to subgenomes

Pairing

A	B	D
T. urartu	*Ae. speltoides*	*Ae. tauschii*
1A	1B	1D
2A	2B	2D
3A	3B	3D
4A	4B	4D
5A	5B	5D
6A	6B	6D
7A	7B	7D

AABBDD monosomic (42-1) × AABB

- If monosomic for D: (A^7 B^7 D^6) × (A^7 B^7) → 14 II + 6 I
- If monosomic for A or B: (A^7 B^6 D^7) or (A^6 B^7 D^7) × (A^7 B^7) → 13 II + 8 I

Using this, determined that chromosomes formerly known as #15-21 belonged to the D genome, while chromosomes #1-14 were from the A and B.

To separate A from B:

- Artificially derived AADD (AA × DD → AD → AADD)
- However, the pairing of the amphidiploid was too irregular to be useful

Used a telosomic series of wheat (gave 20 II + 1 telosome)

Thus, if the telosome belonged to A:

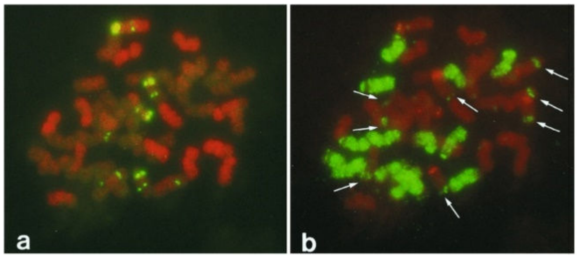
- 20 II + telo A × A^7D^7 → unequal bivalent

If the telosome was from B:

- 20 II + telo B × A^7D^7 → unpaired telosome

Landmarks + GISH

Linares et al., 1998



Use of GISH– Genomic In Situ Hybridization to separate genomes in oat

Oat: 2n = 6x = 42, AACCDD

Based on the premise that each genome has repetitive DNA sequences unique to it

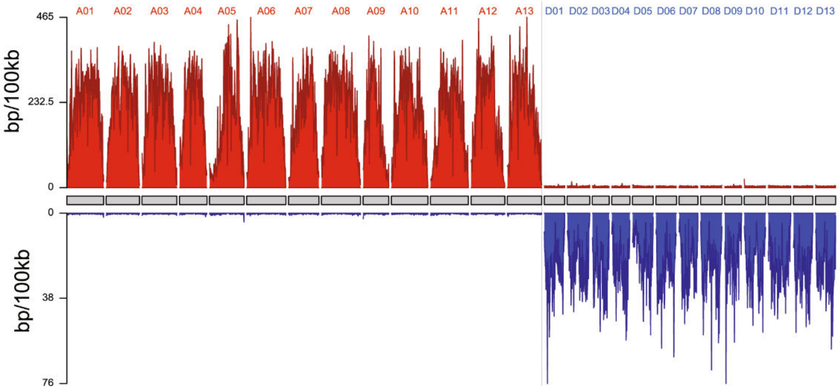
a) *Green = digoxigenin-labeled pTa794 (rDNA, bright spots in bw); orange = rhodamine-labeled pAs120 (medium gray in bw) from the A genome. Unlabeled chromosomes appear dark gray*

b) *Same cell - Green = digoxigenin-labeled pAm1 (C genome) rhodamine-labeled pTa71 (rDNA-specific).*

Transposon insertions

Session and Rokhshar, 2023

- Specific insertions shared between parents and allo4x



Substitution lines

Ising, 1966

1st report of chromosome substitution was in ifafa lily hybrids, 2n = 16

- F1 was doubled with colchicine
- Got “compensated aneuploids” ie, 2n -1 +1
- If the chromosome was from the same morphological group (ie, homoeologue), they got “Pseudo euploids”

Cyrtanthus parviflorus* × *C. mackenii var cooperi.
https://i.pinimg.com/736x/6b/b7/45/6bb745fbbf3f135bde6cbc3e1c203f35.jpg & Wikipedia Commons

Deriving substitution lines

Sears, 1969

BCing to a monosome moves the monosome to a new genotype

E.g., the transfer of chromosome 6B from 'Timstein' into 'Thatcher'

Thatcher (recipient) × (donor) Timstein
(6B nullisomic)
↓
× hybrid monosomic for 6B
↓
×
× ↓
↓
Thatcher with one 6B from Timstein
↓ ⊗
Thatcher with two 6B from Timstein = substitution line

Using substitution lines

Eg, **Berke et al., 1992**

Substitution lines are used to identify genes and assign them to specific chromosomes

- If substitution results in a change of alleles, those genes are on the substituted chromosome

Below is a partial table of reciprocal substitutions between 'Wichita' and 'Cheyenne'

- Data are in terms of deviation from mean of the parent

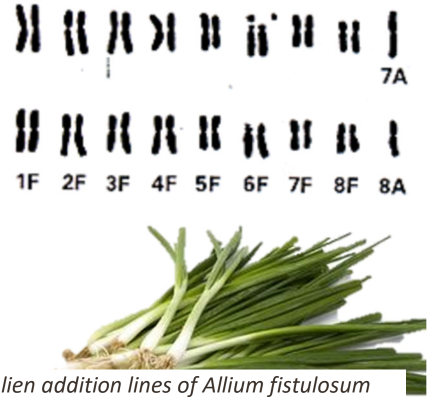
	Wichita chromosomes in Cheyenne background		Cheyenne chromosomes in Wichita background	
Chromosome	Seed Yield (Mg ha^{-1})	Height (cm)	Seed Yield (Mg ha^{-1})	Height (cm)
1A	0.17	1.6	-0.12	-2.4
3A	0.45*	-7.6*	-0.45*	2.6
6A	0.33*	-2.9*	-0.61*	-.01
2B	-0.01	-0.2	0.06	5.1*
3B	-0.62*	0.0	-0.09	-3.9*
5D	0.12	0.1	0.04	1.0

WI3A has a QTL that increases yield, while CNN3A has one that decreases it

Monosomic alien addition lines

Plants that have a single chromosome from another species in addition to their normal chromosomal complement

- Derived from interspecific crosses, followed by repeated backcrossing
- Disomic addition lines → the monosome has a pairing partner



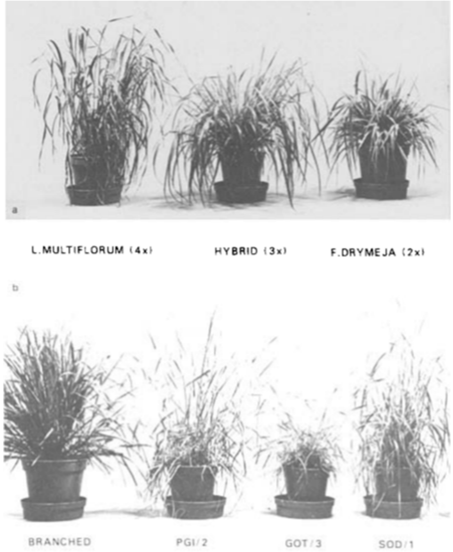
Alien addition lines of Allium fistulosum with chromosomes from A. cepa. From Sigyo et al., 1996. Genes Genet. Syst. 71:363-371

Using addition lines

Distinguish effects of specific alien chromosomes

Eg, **Morgan, 1991**

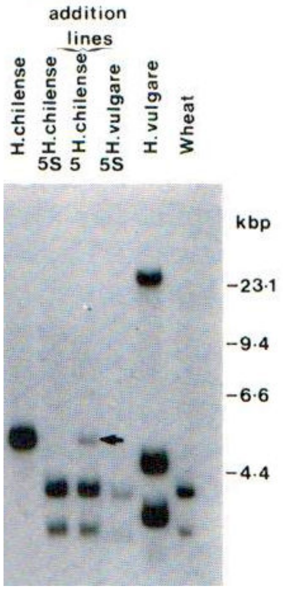
- Effect of 4 different *Festuca drymaja* chromosomes in *Lolium multiflorum*



Detect homoeologies between chromosomes of different species

Assign genes to chromosomes

Principle: If addition line has enzyme or DNA sequence specific to donor species, then that locus is on the alien chromosome



Eg, Cannell et al., 1992

Addition lines of wheat with 5S or 5S·5L of barley

- Probed for ß-hordothionein
- Got band only if 5S·5L was present
- Never got the band in wheat or if only 5S was present
- Therefore, must be on 5L of barley

Limitations to alien addition/substitution lines

Jiang et al., 1994

- Added chromosome causes sterility (e.g., barley 5 into wheat)
- Presence of gametocidal genes on alien chromosome
- Preferential elimination of certain chromosomes
- Tendency of univalents to break
- Poor transmission through the pollen

Previous use of addition lines to study homology via pairing is being replaced with molecular markers

- Works best if determine homology prior to substitution

Using addition line translocations in breeding

Ln-9 gene for leaf rust resistance from *Aegilops umbellulata* to wheat

Eg, Sears, 1956


Leaf rust and spikes of wheat cv. 'Chinese Spring' and of Aegilops umbellulata.

A. umbellulata is a wild grass from the Mediterranean, with 2n = 2x = 14. It is considered to have a C^U genome.

Wheat is 2n = 6x = 42, consisting of A, B, and D genomes

A. *umbellulata* will not cross with wheat, but it will cross with *Triticum dicoccoides,* which 2n = 4x = 28, and has the A and B genomes.

From this, an amphidiploid was derived that had the $AABBC^uC^u$ genomes.

- Crossed readily with wheat, cv. Chinese Spring, → $AABBDC^u$ constitution
- The amphiploid x *aestivum* F1 was backcrossed to Chinese Spring, got 47 seeds
- Selected seedlings for resistance to leaf rust, then looked at chromosomes
- Found 2 resistant plants with only 2 *Aegilops* chromosomes
- Also had a single D genome monosome (gives 20 II & 3 I at meiosis)
- One of these was backcrossed to Chinese Spring a second time


L to R: Chinese Spring with 0, 1, and 2 copies of the Aegilops chromosome.

Got 36 offspring, of which 5 were resistant:

- 1 with 2 *Aegilops* monosomes + a wheat monosome (20 II + 3I)
- 3 with 1 *Aegilops* monosome + a wheat monosome (20 II + 2I)
- 1 with 1 *Aegilops* monosome (21 II + 1 I)

This plant was self-pollinated, and got 119 progeny

- 30 were still resistant to leaf rust
  - 28 had an *Aegilops* monosome
  - 1 had an *Aegilops* bivalent
  - 1 had an *Aegilops* isochromosome


L: A univalent from Aegilops; C: An isochromosome from Aegilops; R: A bivalent of the Aegilops chromosome.

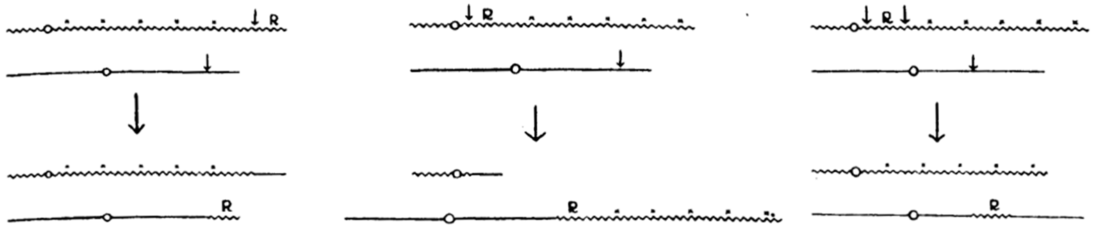
Although the line with the bivalent out of Aegilops could be maintained as a chromosomal addition line resistant to leaf-rust, this is not a viable alternative because other genes on this chromosome have deleterious effects on the wheat.

The ideal would be transfer the resistance gene(s) to wheat, and get rid of all the other parts of the *Aegilops* chromosome

- However, since the *Aegilops* chromosome does not pair with its wheat homoeologue, crossing over will not take place
- Resort to X-rays to induce translocations

However, location of resistance gene(s) on the chromosome was not known

- Several scenarios were possible:



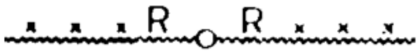
- (left on the diagram) Ideally, resistance is conditioned by a single gene near the telomere. In this case, a translocation will transfer the resistance gene, but few of the deleterious *Aegilops* genes (marked by x's on the diagram).
- (center) If the resistance gene is near the centromere, then a translocation would also transfer considerable *Aegilops* genetic material.
- (right) An interstitial translocation becomes necessary to transfer the resistance gene while minimizing the transfer of *Aegilops* genetic material
    - Interstitial translocations are rare, as more breaks are needed.

Genes on *Aegilops* chromosome hinder transmission through the pollen.

- Therefore, using as male parent selects for the least amount of *Aegilops* chromosome possible

In addition, use of the isochromosome would double the probability of recovering the resistance gene:

- Therefore, prior to meiosis, irradiated the plants having the isochromosome



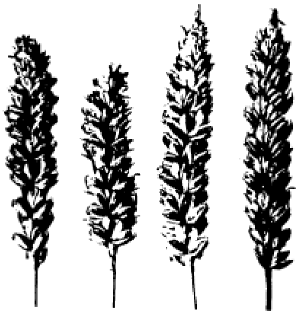
Pollen from irradiated plants was used to pollinate Chinese Spring

- Grew out 6000 plants
    - 132 (2.2% of total) seedlings were rust resistant
    - 92 had the Aegilops isochromosome or a telocentric derivative from it
    - 40 had no intact Aegilops chromosomes, meaning resistance gene must have translocated onto a wheat chromosome

  - 28 of these had an extra chromosome, consisting of part Aegilops, part wheat (i.e., were reciprocal translocations)
  - 12 were initially thought to be intercalary translocations
  - 5 had high transmission rates through the pollen

Abnormalities in the wheat chromosomes were also induced by the X-rays

The final product was distinguishable from Chinese Spring only by its rust-resistance and slightly later maturity.



L-R: Chinese Spring; homozygous for reciprocal (middle pair) and interstitial (right) translocations.

Friebe et al, 1996

The resulting cultivar was called ‘Transfer’

- Terminal transfer of a $6C^{U}L$ segment to 6BL
- Used in some soft-red winter wheats - eg, Arthur 71
- Not widely used due to reduced yield
- Leaf rust overcame the Lr9 gene in 1971

There have been 57 wheat-alien translocations

- 10 are whole arms with breakpoint at centromere
- 45 were terminal translocations
- 2 were intercalary translocations

Most desirable are translocations between homoeologues

- Translocations between non-homoeologues lead to duplications and deficiencies which in turn cause problems in breeding

More on translocations

Jiang et al, 1994

Other ways to induce translocations

- Spontaneous (e.g., 'August') ¿Due to transposable elements?
- Centromeric break and reunion of univalents
- Tissue culture
- Gametocidal genes

In general, few translocations are in use:

- Most alien segments do not compensate well for the lost wheat chromatin
- Linkage drag from alien segment
- Rye 1R has many disease resistance genes, but leads to poor dough quality

Introgression via pairing genes

Review by **Jones et al, 1995**

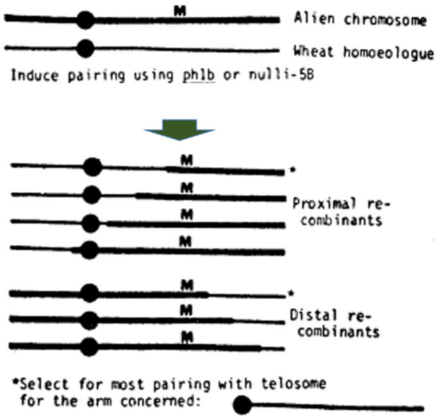
Eliminating the 5B chromosomes is a way to get crossing over between the A, B, and D genomes. This has also helped transfer traits into wheat from wild species.

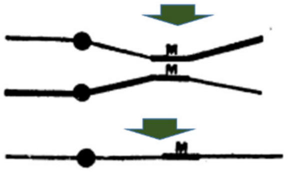
- The alternative is to use translocations

The following diagram illustrates the introgression of a gene from a related species by crossing over in a *phlb phlb* background

- Used by Sears (1978) to transfer the Lr24 gene for resistance to leaf rust from *Agropyron elongatum*
- Also used by Riley et al., 1968, to transfer resistance to yellow rust from *Aegilops comosa* (MM genome)

Sears 1983

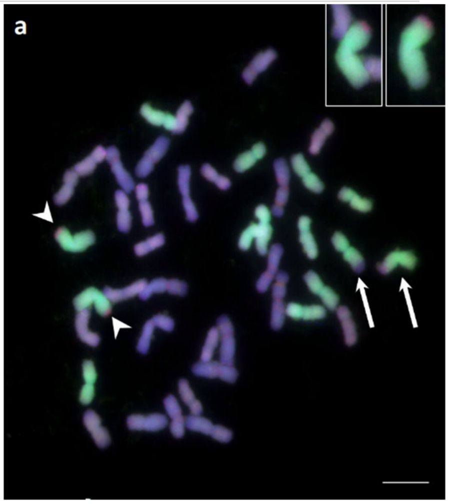




- Need to point out that rather than pairing, GISH or markers are used now.
- Also, the size of the introgression can now be monitored with molecular markers.
- CRISPR is not yet a substitute

Monitoring with GISH

Eg, **Gao et al., 2021**



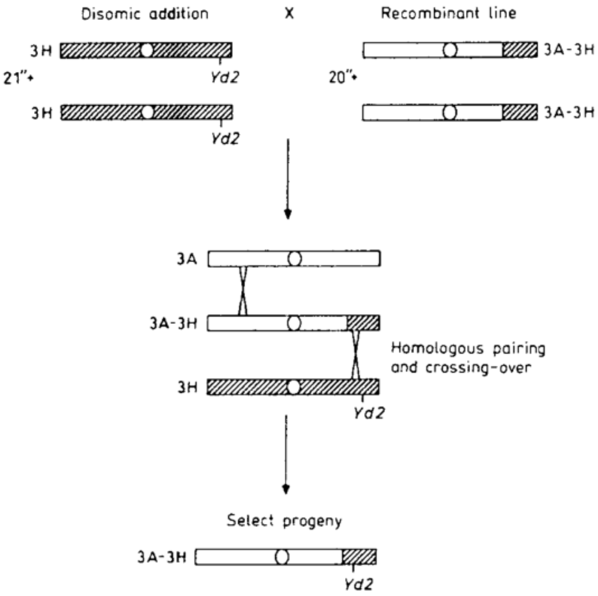
Segment from 2N^{v}S *Aegilops ventricosa* translocated on to 2AS

- 33-Mb = 6.5% of 'Jagger' = larger than most rice chromosomes
- Contains 535 gene models
- 10% are disease resistance genes
- In 80% of Kansas & CIMMYT ones

  - *Red- N genome from Aegilops uniaristata*
  - *Green – A genome probe from T. urartu*
  - *Insert: chromosome 2A*
  - *Arrowheads → 2N^{v}S-2AS translocation*
  - *Arrows – ancestral translocation between 4AL and &BS*
  - *Bar – 10 µm*

Continued introgressions

Islam & Shepherd, 1992



Once a segment has been introgressed from one species to another

- It can continue to be used to introgress any genes on that particular segment

LIMITATIONS

- Not effective to get wheat/rye pairing: must use X-ray translocations
- Ph2 gene on 3DS: doesn't affect wheat, but does affect hybrids