PROGRESS ON PHYSICAL MAP OF WHEAT CHROMOSOME 5A

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CONSIGLIO PER LA RICERCA
E LA SPERIMENTAZIONE
IN AGRICOLTURA
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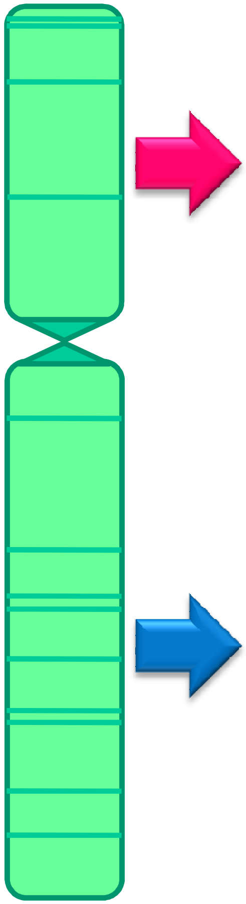
PHYSICAL MAP OF WHEAT CHROMOSOME 5A



5AS
(295Mb)

5AS BAC library ”TaaCsp5AShA”

46.080 clones (120 plates)

mean insert size = 120kb

5AS coverage= 16.5X

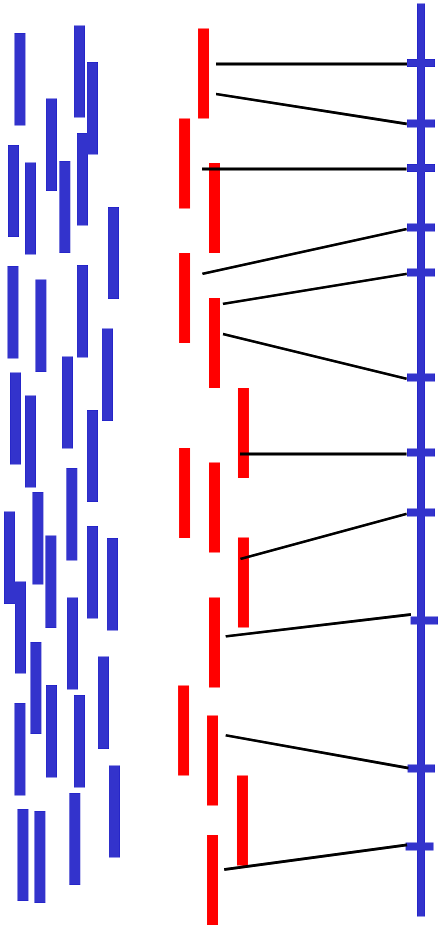
5AL
(532Mb)

5AL BAC library ”TaaCsp5ALhA”

90.240 clones (235 plates)

mean insert size = 123kb

5AS coverage= 18.3X



BAC Library Fingerprinting and Contig Assembly

BAC fingerprinting

✓ All BAC clones were fingerprinted using SnaPshot method.

✓ 75,995 high quality fingerprints after contamination removal.

Useful fingerprints were assembled using FPC according to IWGSC rule and a first MTP (MTP-FPC) was established after automated assembly.

✓ Several successive cutoffs were used from 1e-75 to 1e-25

✓1,308 contigs for 5AS

✓2,556 contigs for 5AL

	5AS		5AL	
CHROMOSOME ARM SIZE (Mbp)	295		532	
# FINGERPRINTED CLONES	44.740		51.072	
INSERT SIZE (Kbp)	120		123	
COVERAGE	16.5X		10.4X	
ASSEMBLY METHOD	FPC	LTC	FPC	LTC
ASSEMBLY STRINGENCY	10^{-45}	10^{-20}	10^{-45}	10^{-15}
# USEFUL FINGERPRINTS	36.165 (80.8%)		39.830 (76.0%)	
# CLONES IN CONTIGS	25,084	26,659	27,764	29,610
# SINGLETONS	11,081	9,506	12.066	10.220
# MTP CONTIGS	1,308	652	2.556	1.504
# MTP CLONES	4,201	5,412	6.560	8.709
ESTIMATED CHR COVERAGE (%)[a,c]	106	---	103	---
CONTIGS N50 (Kb)[b,c]	271	---	229	---
CONTIGS L50 (Kb)[b]	353	---	822	---

a: based on the total number of contigs

b: based on a CB unit size of 1.2 kb for SnaPshot

c: based on the expected size of the chromosome arm

The same useful fingerprints were also assembled using LTC according to Frenkel et al. 2010 guidelines and a second MTP (MTP-LTC) was established after automated assembly

✓652 contigs for 5AS

✓1,504 contigs for 5AL

3D Pools of 5A MTP (FPC- and LTC-)

The MTP clones are stored in 384-well plates:

MTP-FPC: 11 for 5AS and 18 for 5AL

MTP-LTC: 15 for 5AS and 23 for 5AL

Picking of 10,761 clones

5AS		**5AL**
4,201	+	6,560

DNA extraction

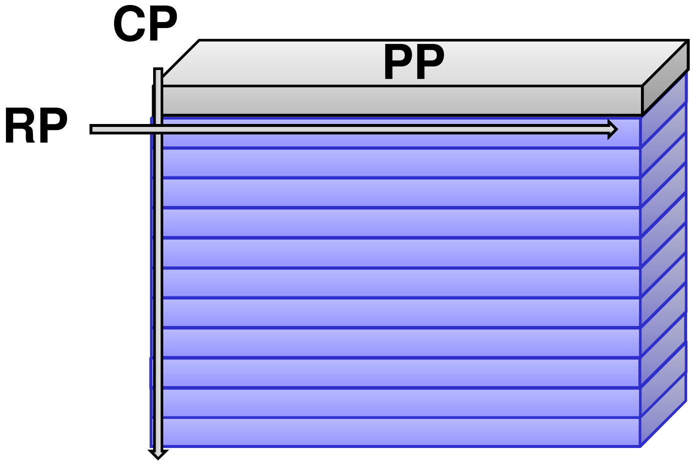
3D-pooling of DNAs

51 analysis 58 analysis

Picking of 14,121 clones

5AS		**5AL**
5,412	+	8,709
55 analysis		63 analysis

3D-pooling of BACs



Genetic Mapping

4 SEGREGATING POPULATIONS:

- 383 F2 Chinese Spring (CS) x Renan (*T. aestivum* x *T. aestivum*)
- 188 RILs from Chinese Spring x CS-*T. dicoccoides* Disomic Substitution 5A TDIC (5A CS) (*T. aestivum* x *T. turgidum dicoccoides*)
- 132 RILs from DV92 x G3116 (*T. monococcum* x *T. monococcum*)
- 124 RILs from Latino x MG5323 (*T. turgidum* ssp *durum* x *T. turgidum* ssp *dicoccum*)

SEVERAL CLASSES OF MOLECULAR MARKERS:

- TE junction-based markers (ISBP, RJM, RJJM) and SSRs from survey sequencing of 5AS
- SSRs and SSR-ESTs from literature
- COSs

The RILs of Latino x MG5323 have also been hybridized with the Illumina 90K chip and about 500 SNPs have been mapped on 5A.

The integration of this map with other 6 durum wheat maps is leading to more than 1000 SNPs mapped on 5A.

Anchoring of FPC-MTPs

Screening of MTP-3D pools using PCR
with all available markers
(SSRs, EST, COS and TE-derived)

↓

anchoring of contigs using a dedicated scripting

↓

179 markers positive for at least one BAC

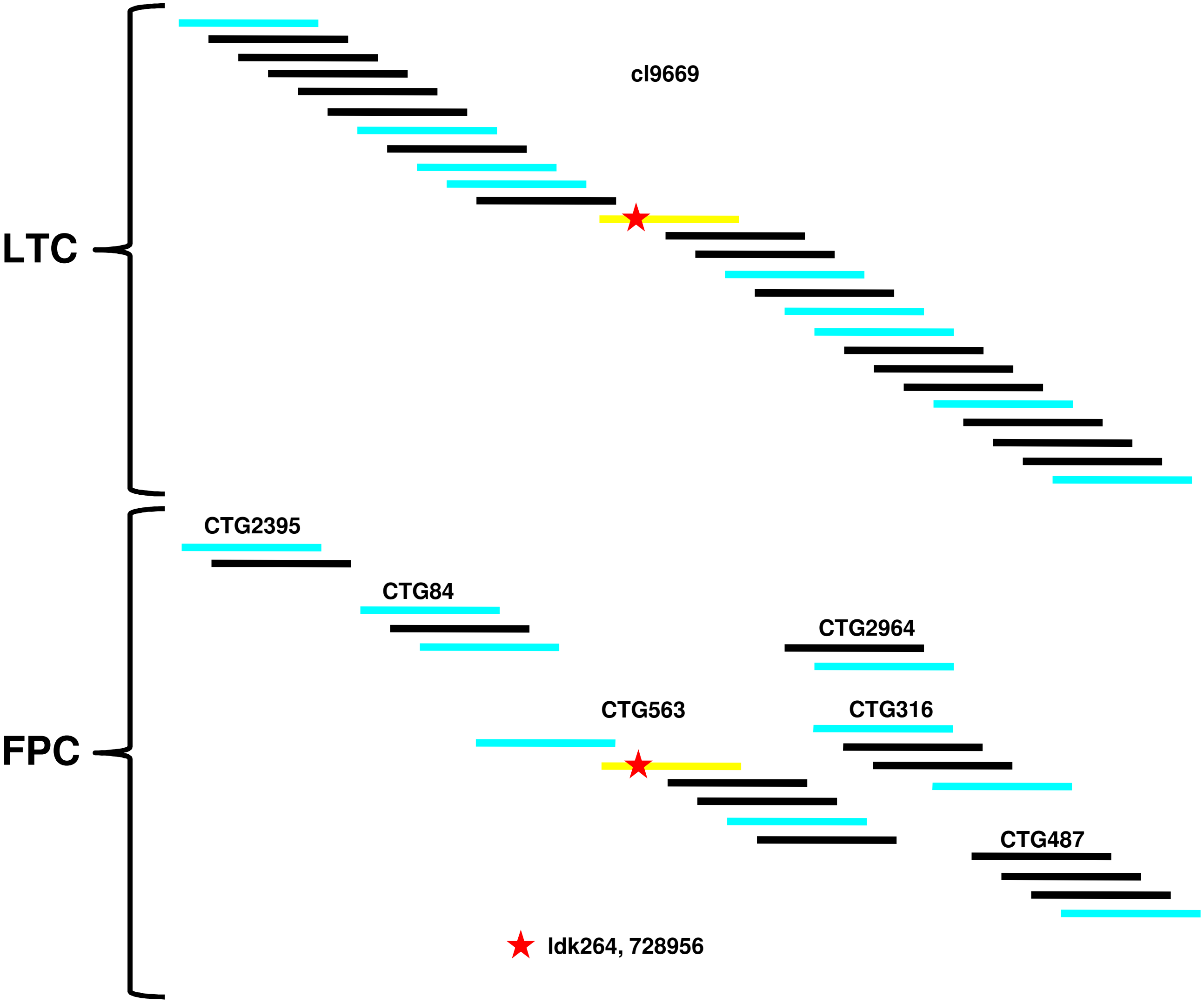
↓

170 contigs anchored by at least one marker

↓

total length anchored:
41 Mb of 5AS and 13 Mb of 5AL

Integration FPC- and LTC-MTPs



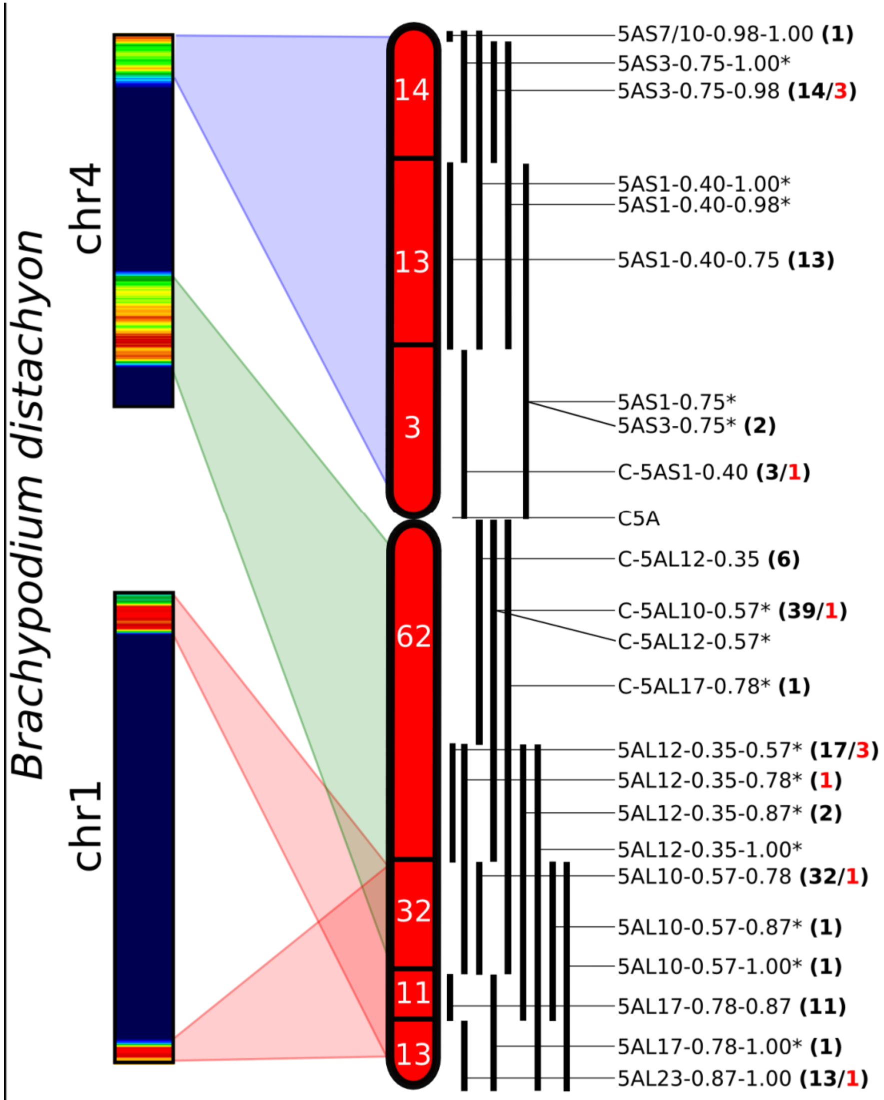
Integration FPC- and LTC-MTPs

Integration between FPC-MTP
and LTC assembly

FPC ASSEMBLY integrated with LTC	5AS	5AL
all anchored markers	134	60
EST and COS	6	10
SSR and SSR-EST	37	44
STS	1	5
TE-based	90	1
FPC unique contigs	122	48
total length anchored (kb)	41,139	12,905
FPC unique contigs after integration	309	96
total length anchored (kb)	96,068	23,744
chromosome percentage	32,6	4,5
chromosome arm size (Mbp)	295	532

5AS: 96 Mb
5AL: 24 Mb

Anchoring of LTC-MTPs



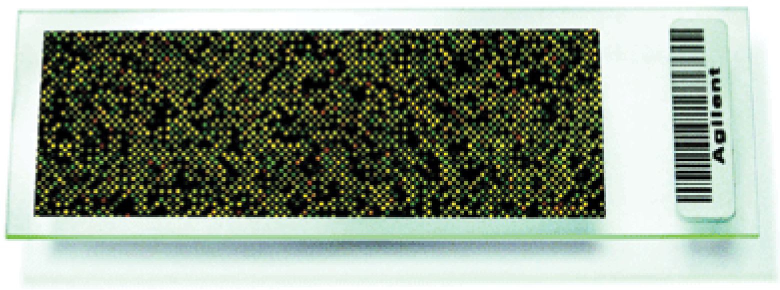
Reference Genome Zipper for 5A

Vitulo et al, PlosOne 2011

A15K Agilent custom microarray was designed
4,722 sequences
deriving from several sources:

- Genome Zipper reads
- 5A ESTs from GrainGenes database
- RFLPs, SNPs, SSRs, TE-based junction markers

12,676 probes have been developed



Hybridizations are underway…

FPC-MTP BAC End Sequencing

5AS-FPC-MTP

2,487 clones recovered
3,862 good quality reads (78%)
representing 2.8 Mb
average length 715 bp

2,307 ISBP markers
72 SSRs (di- tri- tetra-nucleotide)

5AL-FPC-MTP

2,835 clones recovered
5,194 good quality reads (92%)
representing 3.3 Mb
average length 650 bp

2,641 ISBP markers
52 SSRs (di- tri- tetra-nucleotide)

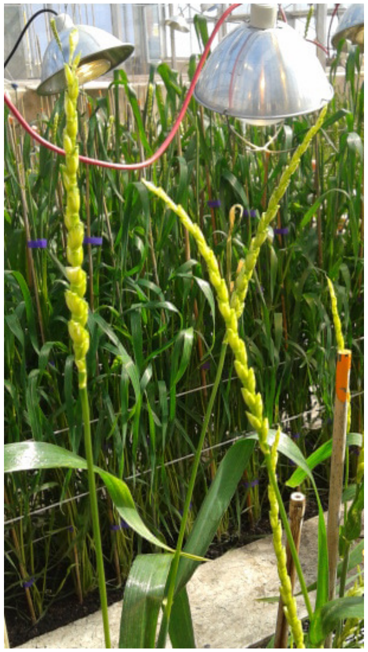
1,964 ISBPs (considering only one markers per sequence)
+
124 SSRs
ready to be mapped onto radiation hybrid panel

A radiation hybrid panel for 5A

Chinese Spring
pollen

X

nulli5Atetra5**B**
nulli5Atetra5**D**



Approx. 390 crosses performed for each nullisomic line

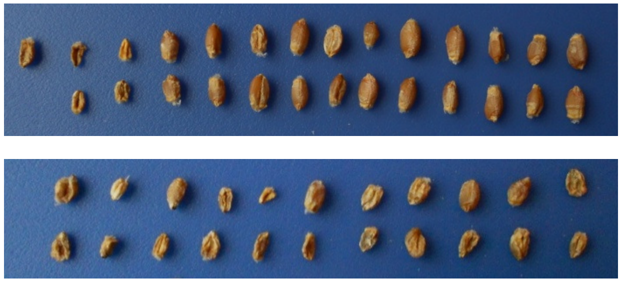
Nulli5Atetra5B → 3500 seeds
Nulli5Atetra5D → 5600 seeds

Nulli5Atetra5 ⊗

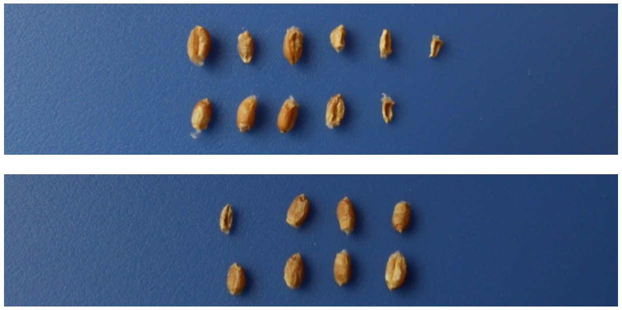
Nulli5Atetra5 ⊗

Nulli5Atetra5B x CS
Nulli5Atetra5D x CS

Nulli5Atetra5B
x
CS 1 Kr



Nulli5Atetra5D
x
CS 1 Kr



Collaborations..

***Yellow rust* (5AL)**

with Jorge Dubcovsky
University of California,
Davis, CA, USA

***Fusarium headblight, FHB* QTL (5AS)**

with Hermann Buerstmayr
Institute for Biotechnology in Plant Production,
Tulln, AUSTRIA

***Yellow mosaic virus (WYMV)* (5AL)**

with Xiue Wang
Nanjing Agricultural University,
Nanjing, CHINA

5AL-4AL breakpoints

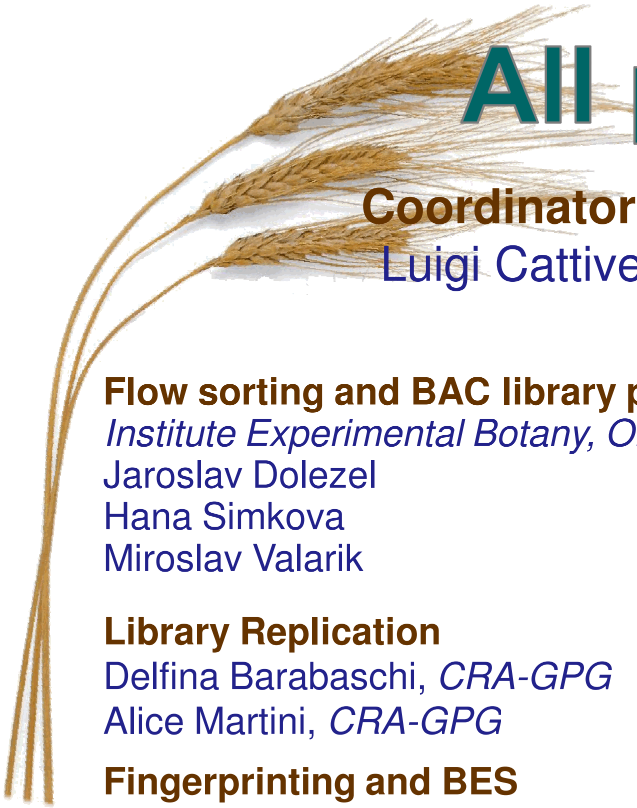
with Chunji Liu
CSIRO Plant Industry, Queensland
Bioscience Precinct, Australia

Oxalate oxidase (5AS)

with Renato D’Ovidio
Universita' degli Studi della Tuscia
Viterbo, ITALY

Outlook

- LTC-MTPs anchoring by hybridization on Agilent array
- Integration between two anchoring data (from FPC and LTC assembly)
- Radiation Hybrid mapping with markers developed from BES
- In silico anchoring with 90K Illumina array data
- End-merging of contigs: manual assembly with anchoring information



All people involved

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Flow sorting and BAC library preparation

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Hana Simkova
Miroslav Valarik

Library Replication

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Alice Martini, *CRA-GPG*

Fingerprinting and BES

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Federica Cattonaro
Federica Magni
Simone Scalabrin

Genetic Mapping

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Radiation Hybrid panel

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Ajay Kumar*, NDSU, USA*

Citogentic Mapping

UniBari, Italy
Agata Gadaleta
Antonio Blanco

Anchoring Genetic to Physical

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Survey Sequencing

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