UNIVERSITÀ DEGLI STUDI DELLA Tuscia

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DEPT. OF AGRICULTURE AND FOREST SCIENCES

Impact on Durum Wheat of Small Introgressions from Wild *Thinopyrum* Species conferring Effective Resistance to *Fusarium* Diseases: Breeding performance and Metabolic Responses

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Challenges for crop species like DW to "produce more with less"

- **FOOD SECURITY** issue → in the next 20-30 years, need to **produce** as much **food** as has been consumed over the entire human history
- **FOOD SAFETY** issue → take care of food health and nutritional value
- Need to reach these goals **SUSTAINABLY**, in the face of several hindrances, including:
  - **decreasing WATER + ARABLE LAND**
  - **ENVIRONMENTAL DETERIORATION**
  - **CLIMATE CHANGES**

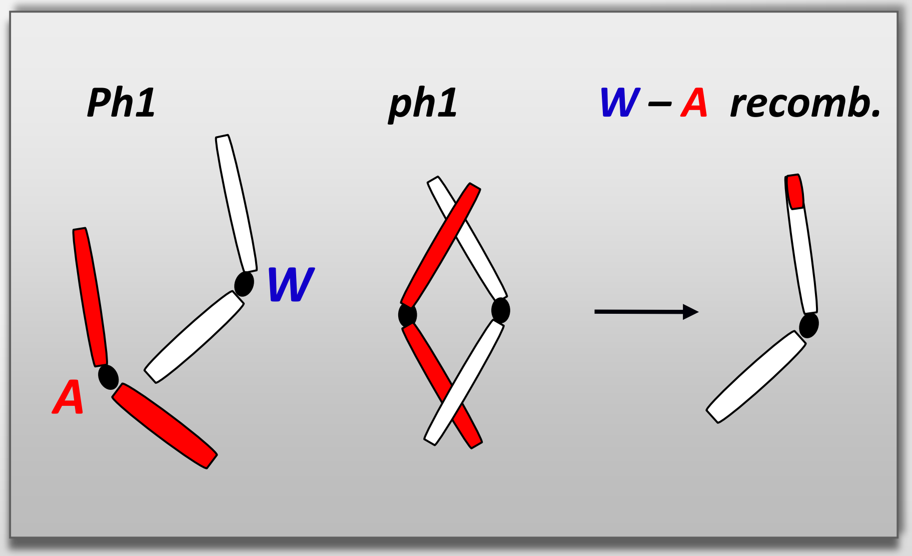
Sustainable strategy → increase genetic variability

Domestication & Intensive Breeding

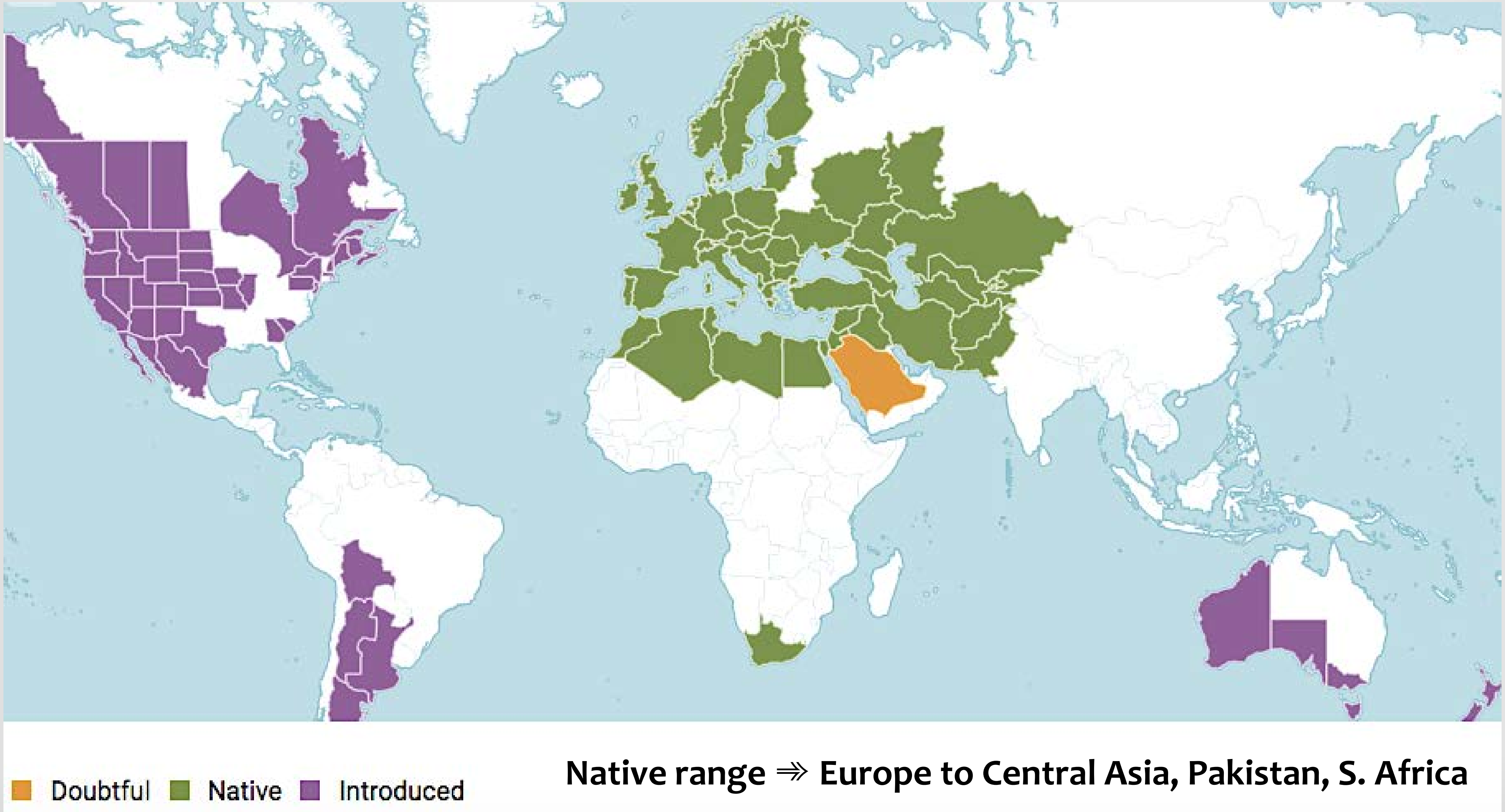
Wheat **Chromosome Engineering** (CE)

CE is a complex cytogenetic approach enabling the ***"…transfer of segments of alien (A) chromosomes carrying particular desired genes to wheat (W) chromosomes…"*** (Sears, 1972)

- Alien species include a vast array of **wild wheat relatives**, **a rich source of still untapped variability**, which can be exploited through CE to enrich the crop genetic base, even resorting to less closely related species (homoeologous chromosomes)
- **Homoeologous pairing and recombination-based CE** (mainly through use of ***Ph1* wheat mutants**), coupled with modern selection methods, allows attainment of largely **balanced products** and introgression of alien chromosome **segments of minimal size**, essential requisite for stability and practical usefulness of transfer products, particularly for **4x DW**, compared with 6x bread wheat



Chromosome engineering to transfer into durum wheat (DW) useful genes from tertiary genepool: the case of *Thinopyrum* genus



Native range ⇛ Europe to Central Asia, Pakistan, S. Africa

Focus on group 7 genes /QTL

Thinopyrum ponticum

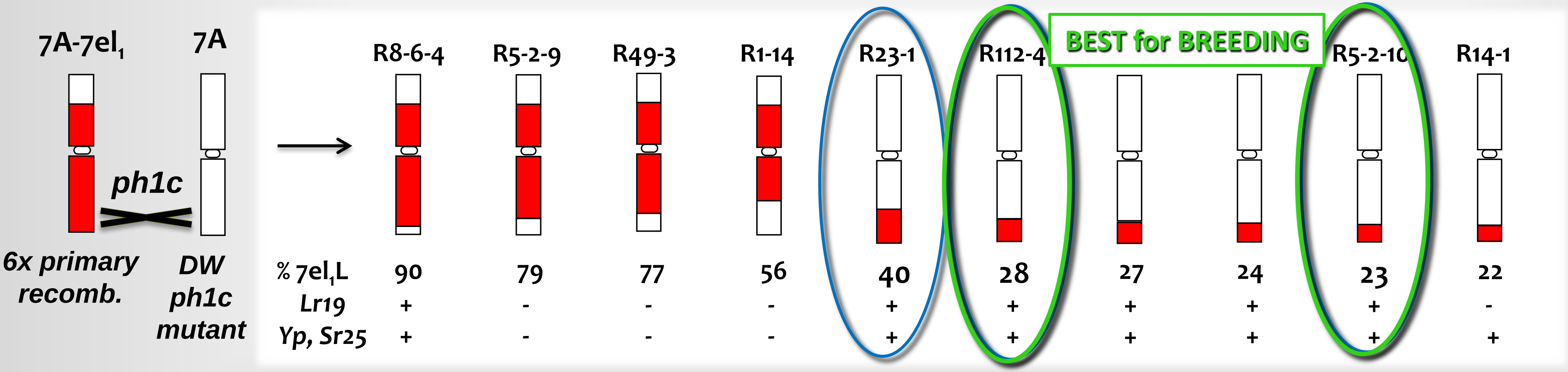
2n=10x=70

7el$_1$ chromosome

7el$_1$L-7AL transfers

Ceoloni et al. 2005
Gennaro et al. 2009

Kuzmanović et al. 2014, 2016, 2018



	R8-6-4	R5-2-9	R49-3	R1-14	R23-1	R112-4			R5-2-10	R14-1
% 7el$_1$L	90	79	77	56	40	28	27	24	23	22
Lr19	+	-	-	-	+	+	+	+	+	-
Yp, Sr25	+	-	-	-	+	+	+	+	+	+

R23 **R112** **R5**

Derivative of R5-2-10 recombinant registered as cv. **CINCINNATO** (2010)

Near-isogenic recombinant lines (NIRLs) in DW cv. Simeto

A weak point of the DW 7A-7el1 recombinants: NO tolerance to *Fusarium* diseases

- For **DW**, both Fusarium Head Blight (**FHB**) and Fusarium Crown Rot (**FCR**) are extremely dangerous diseases, against which the species has **no effective defense**

FHB

- Typically occurs in environments with **humid and warm conditions** around flowering stage
- Main causal agent: *F. graminearum*

FCR

- Prevails under **water-deficit and hot conditions** during flowering time and grain-filling
- Main causal agents: *F. culmorum, F. pseudograminearum*

- Both diseases can dramatically reduce the crop value, being responsible for **grain yield reduction**, and for production of health dangerous **mycotoxins** (e.g. DON), a particularly undesirable aspect for DW, mostly used for transformation into human food products
- In wheat germplasm, QTLs for FHB and FCR resistance appear to have independent location

Enriching 7A-7el$_1$ DW recombinants with FHB resistance : a “nesting” approach

1. Exploiting *Fhb7* from *Th. ponticum* 7el$_2$

🟧 7el$_1$ (= 7Ag)

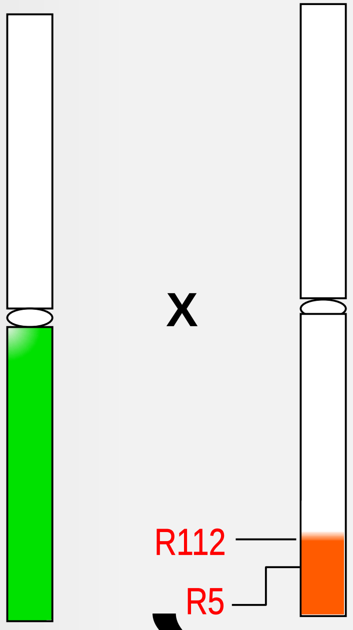
🟩 7el$_2$

▨ 7el$_1$ + 7el$_2$

KS24
6x
7DS.7el$_2$L

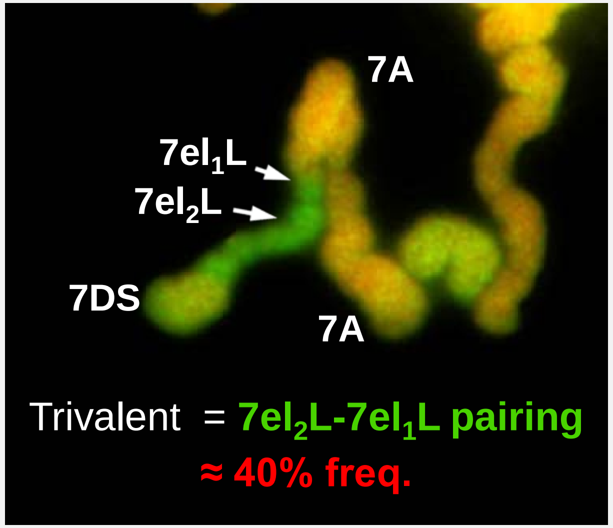
X

R5/R112
4x
7AS.7AL-7el$_1$L



	KS24	R5/R112
FHB res.* *(Fhb7)	+	–
Lr19, Sr25	–	+
Yp	+	+

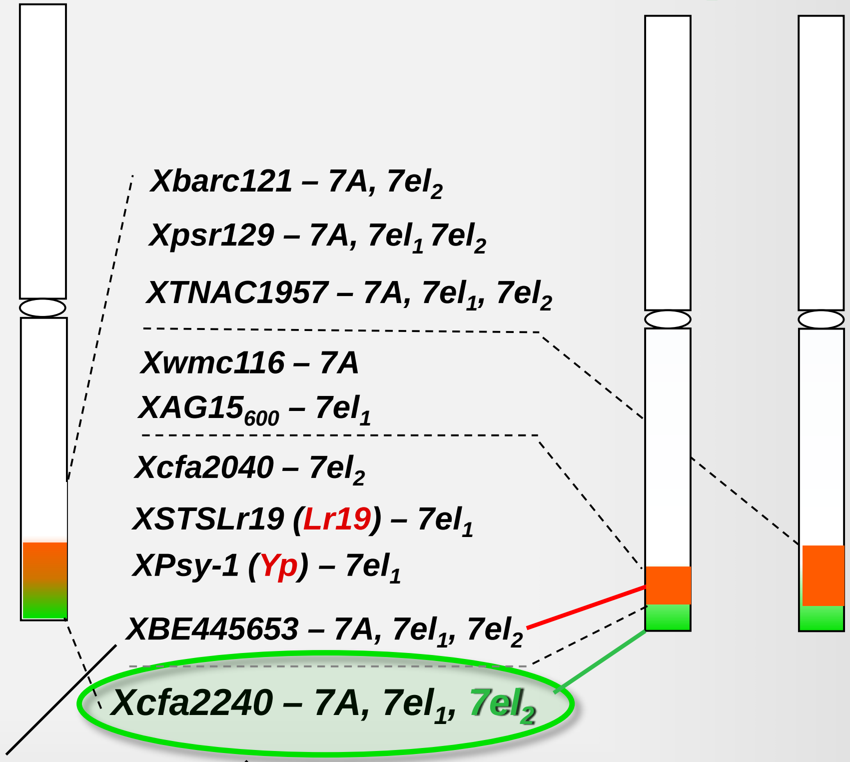
5x F$_1$



Trivalent = **7el$_2$L-7el$_1$L pairing**
≈ 40% freq.

BCs to durum
➔ select 7A
recomb. types

R216 (R5+7el$_2$) **R193** (R112+7el$_2$)



Xbarc121 – 7A, 7el$_2$
Xpsr129 – 7A, 7el$_1$ 7el$_2$
XTNAC1957 – 7A, 7el$_1$, 7el$_2$

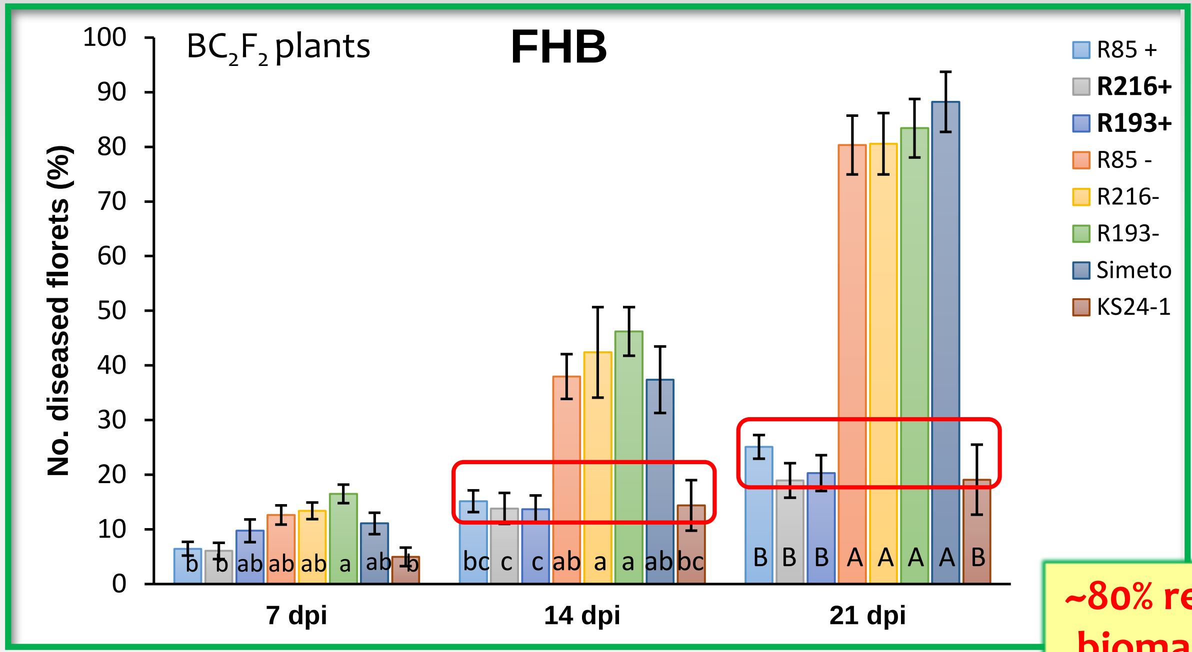
Xwmc116 – 7A
XAG15$_{600}$ – 7el$_1$

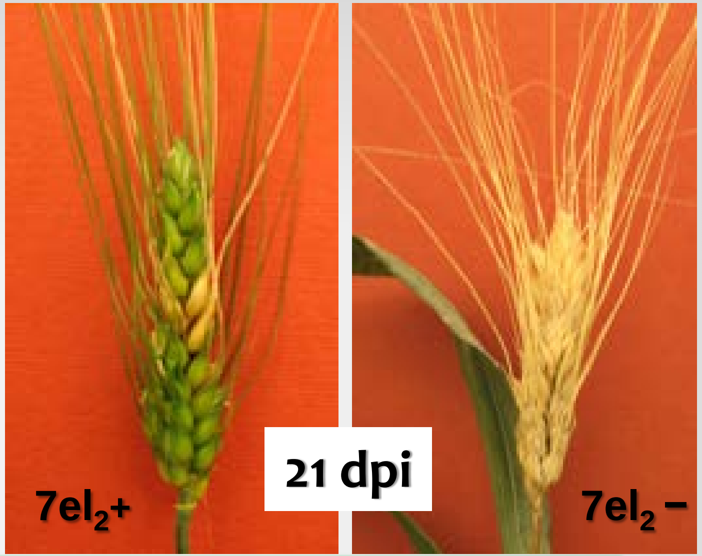
Xcfa2040 – 7el$_2$
XSTSLr19 (Lr19) – 7el$_1$
XPsy-1 (Yp) – 7el$_1$
XBE445653 – 7A, 7el$_1$, 7el$_2$
Xcfa2240 – 7A, 7el$_1$, 7el$_2$

flanking ***Fhb7*** locus
(Shen & Ohm, 2007)

Forte *et al., Mol Breeding* 2014;
Ceoloni *et al., Crop & Pasture Sci* 2014

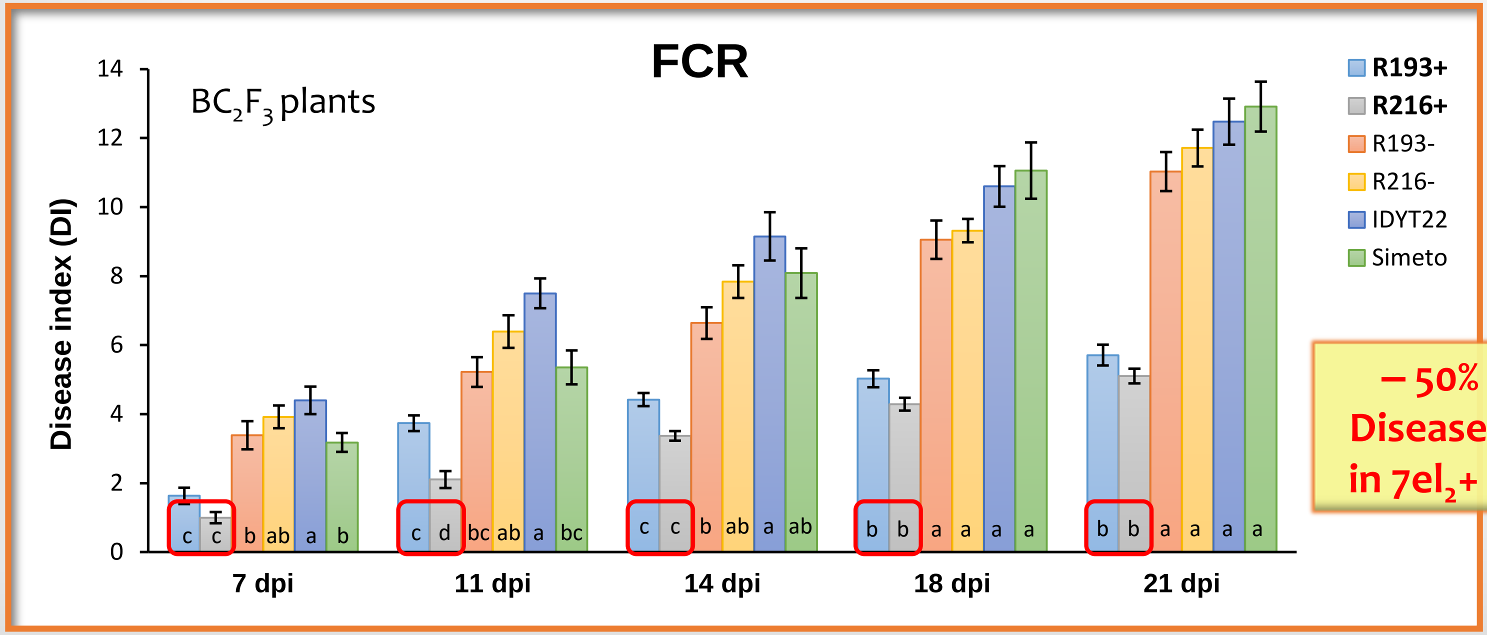
F. graminearum
point-inoculation





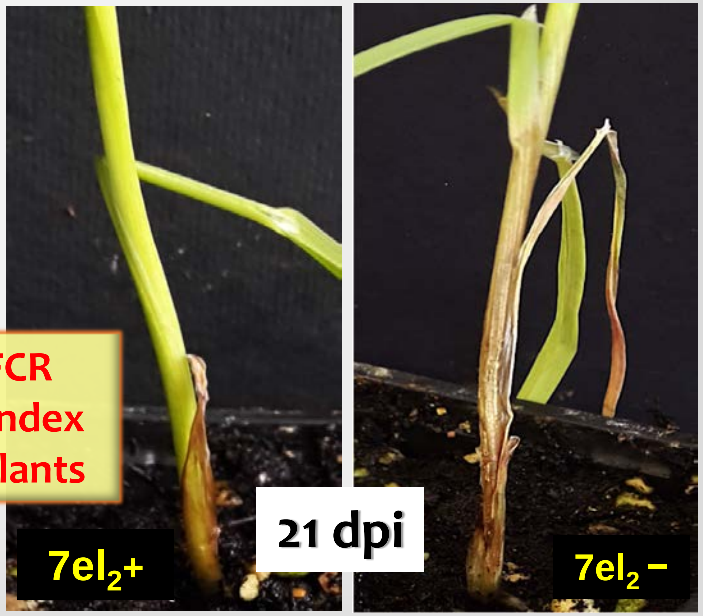
~80% reduction of FHB severity and fungal biomass (*TRI6* expression) in 7el$_2$+ plants

F. culmorum
seedling
inoculation



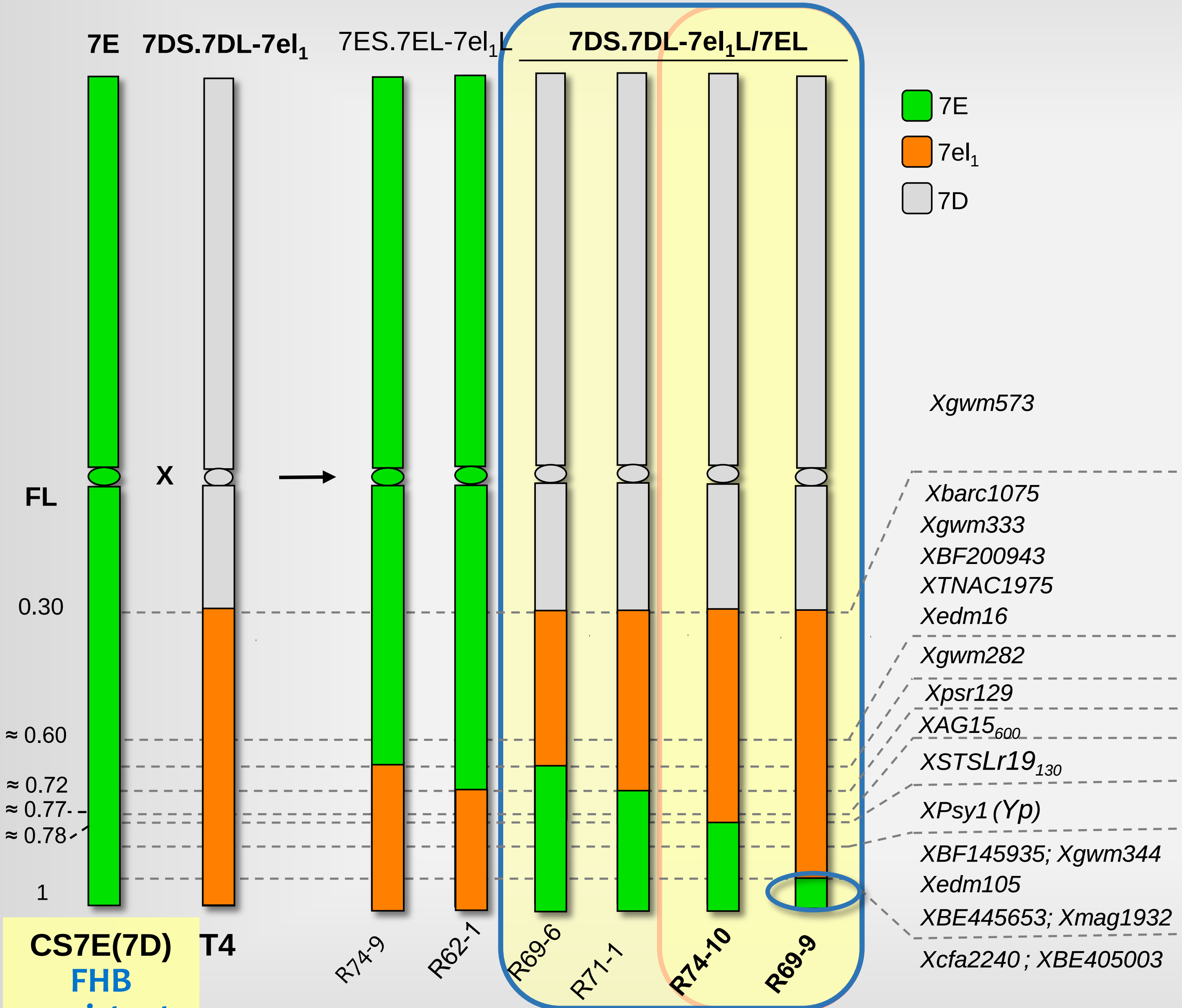
(**DI** = *symptom extension x browning index*)

– 50% FCR Disease Index in 7el$_2$+ plants



Forte et al., *Mol Breed* 2014; Kuzmanović *et al.*, *Frontiers Plant Sci* 2019

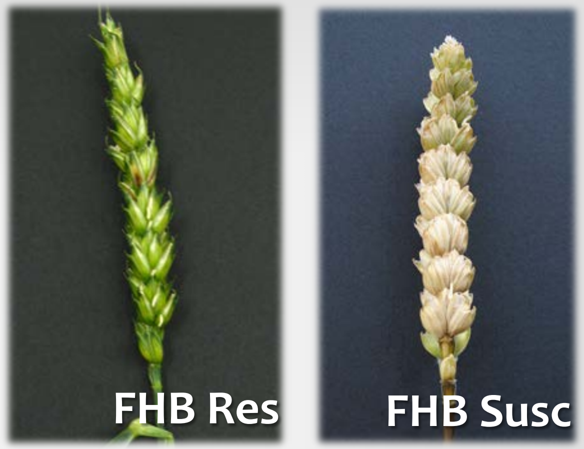
2. Nesting FHB resistance *Th. elongatum 7E* QTL into 7el1L segments (bread wheat)



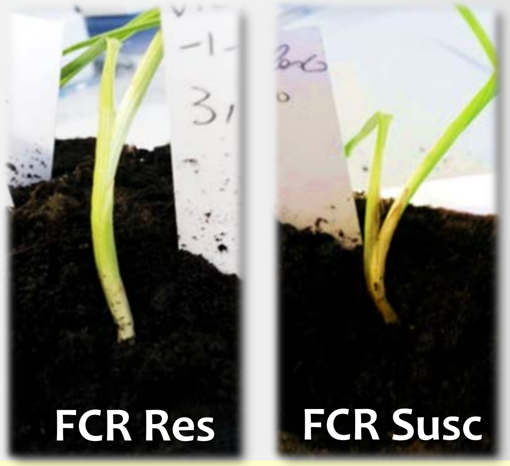
CS7E(7D)
FHB resistant
(Fedak et al.)

FHB & FCR resistant

] ***Fhb-7EL* localization**



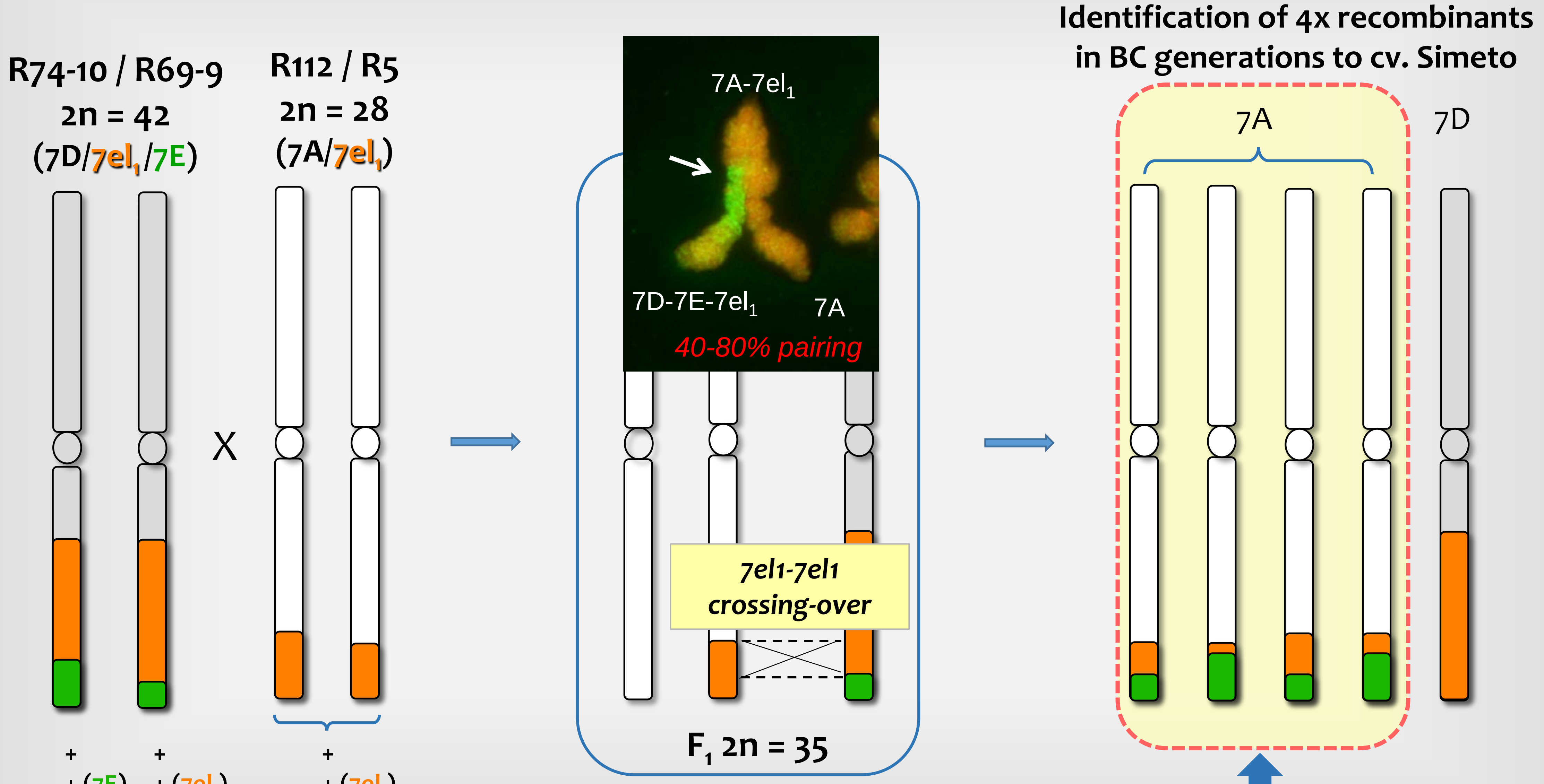
> 90% reduction in FHB and fungal biomass (***TRI6*** **expression) after *F. graminearum* inoculation**



70% reduction in FCR induced by *F. culmorum* e *F. pseudograminearum*

Ceoloni *et al.*, *Theor Appl Genet* 2017

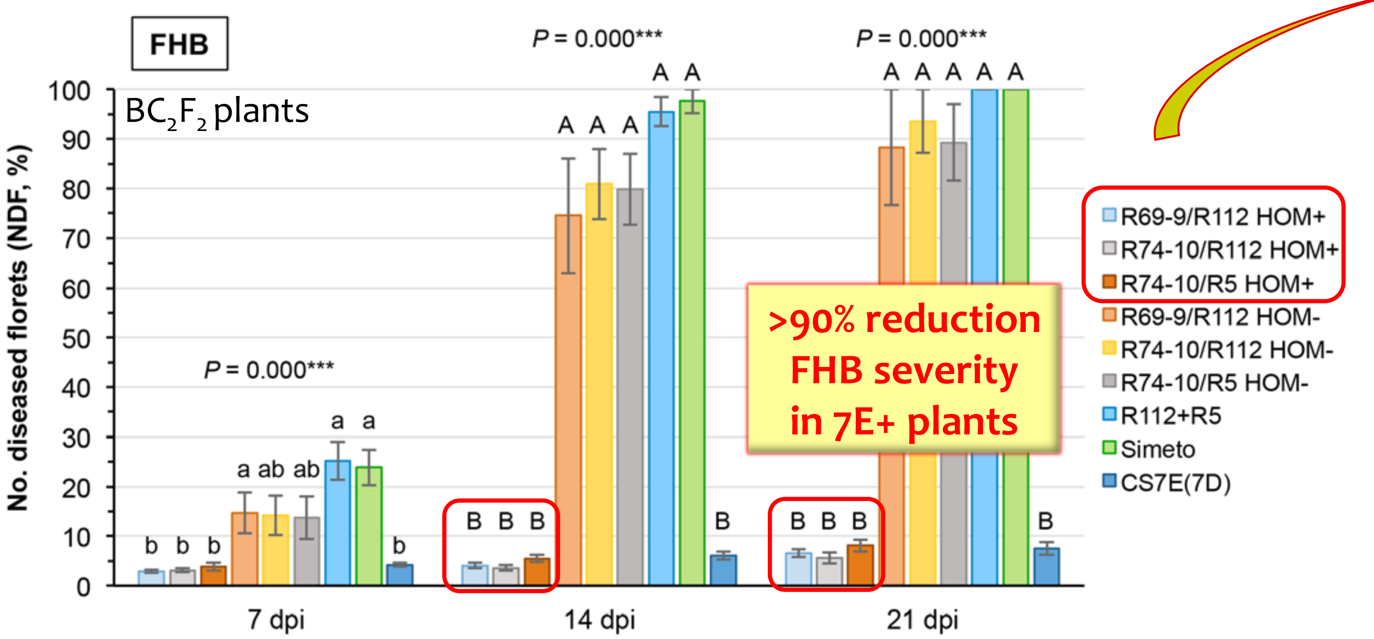
From bread wheat to durum wheat 7el$_1$ + 7E recombinants



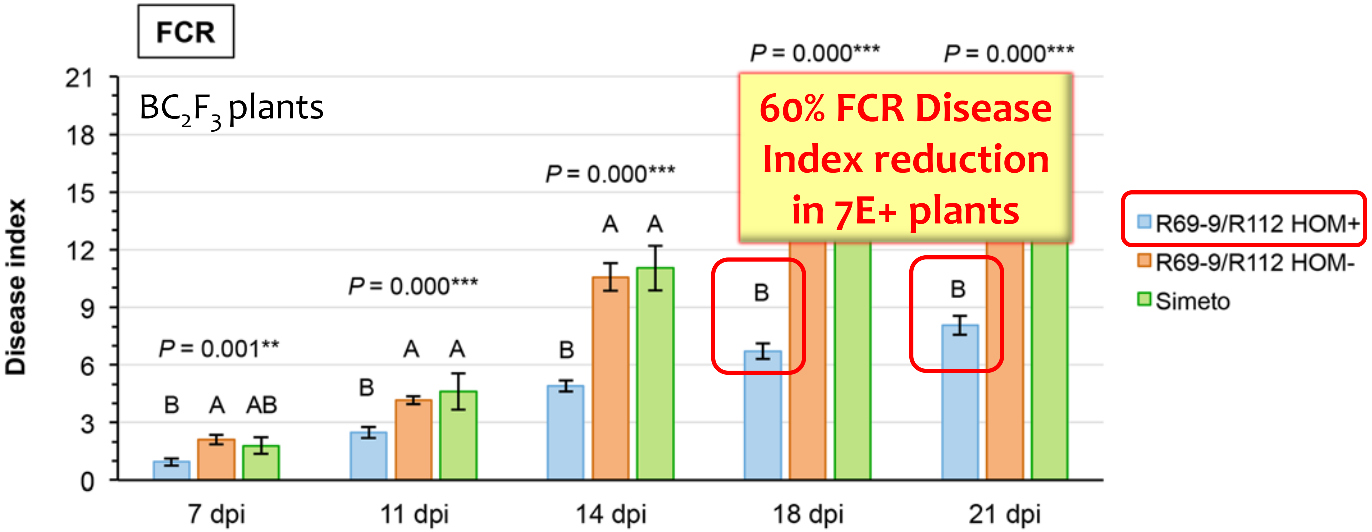
	R74-10	R69-9	R112 / R5
Lr19	+	+	+
Yp	+ (7E)	+ (7el$_1$)	+ (7el$_1$)
Fhb-7E	+	+	-
Yield QTL	+	+	+

Kuzmanović *et al.*, *Frontiers Plant Sci* 2019

MAS & chrom. count



>90% reduction FHB severity in 7E+ plants



60% FCR Disease Index reduction in 7E+ plants

FHB Infected spikes

SUSC. types (HOM-) vs. *Fhb-7EL* HOM+:

- 76% reduction No. seeds
- ≈ 80% reduction TGW

DON & D3G content in wholemeal flour (UHPLC-MS)

Genotype	**DON (ppm)**		**D3G (ppm)**		**D3G/ DON+D3G**	
	M	SE	**M**	SE	**M**	SE
7E HOM + R69-9/R5	**0.25**	0.04	2.82	0.58	0.894	0.02
7E HOM –	**154.11**	4.52	0.72	0.06	0.005	0.00
P value $_{\text{t-test}}$	***		**		***	

800 ×

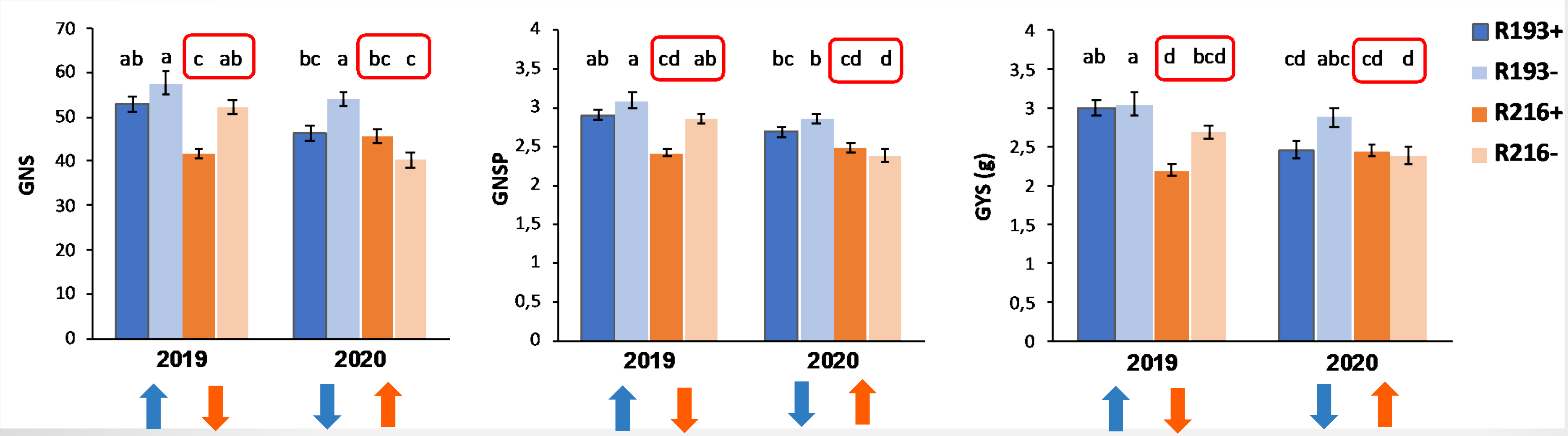
Breeding potential of 7el1+7el2 and 7el1+7E DW recombinants

small scale field trials in 2019 and 2020 seasons in Central Italy

OVERALL MESSAGE: NO MAJOR PENALTY

2019 – rainier and cooler
2020 – drier and hotter

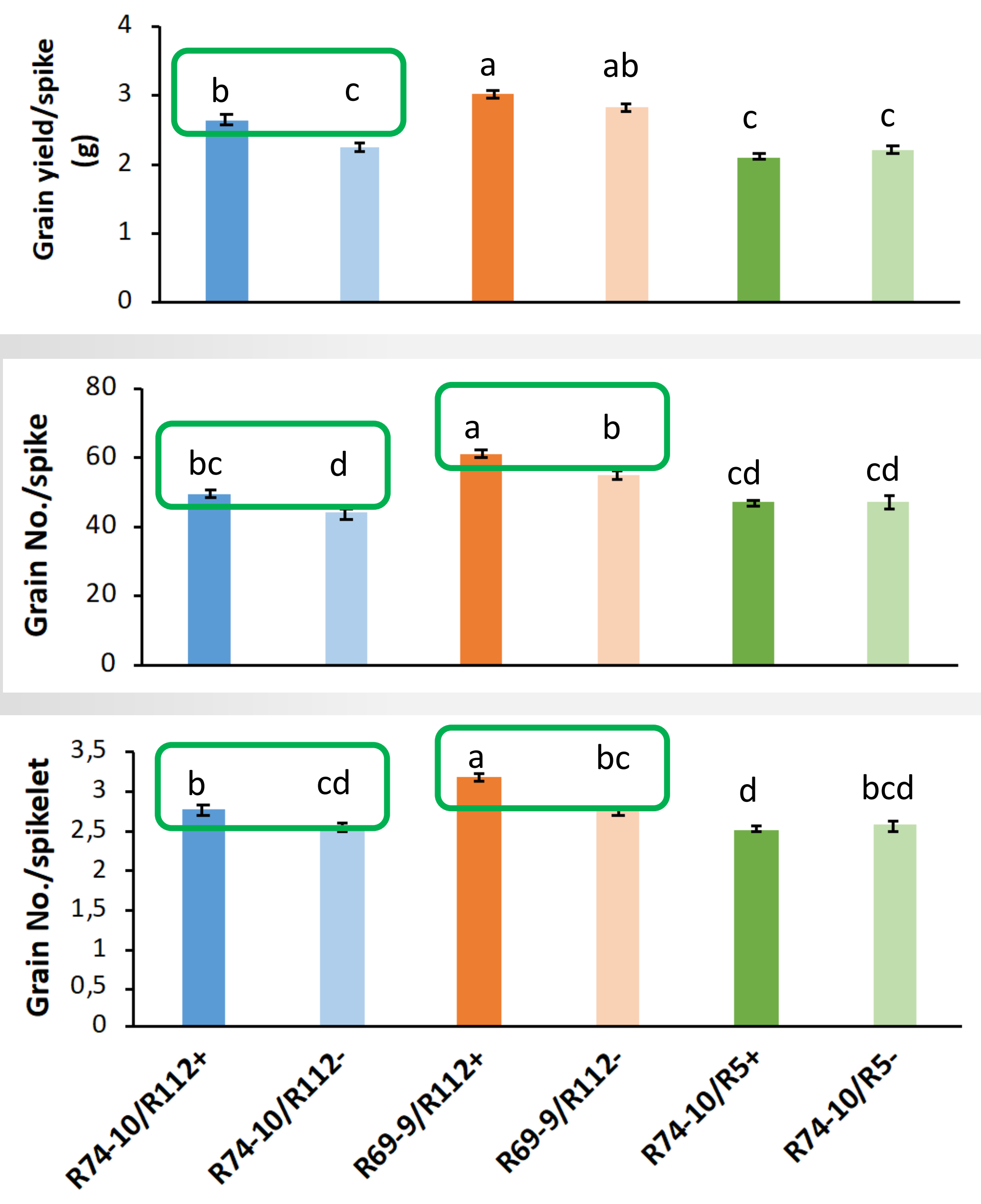
7el1+7el2



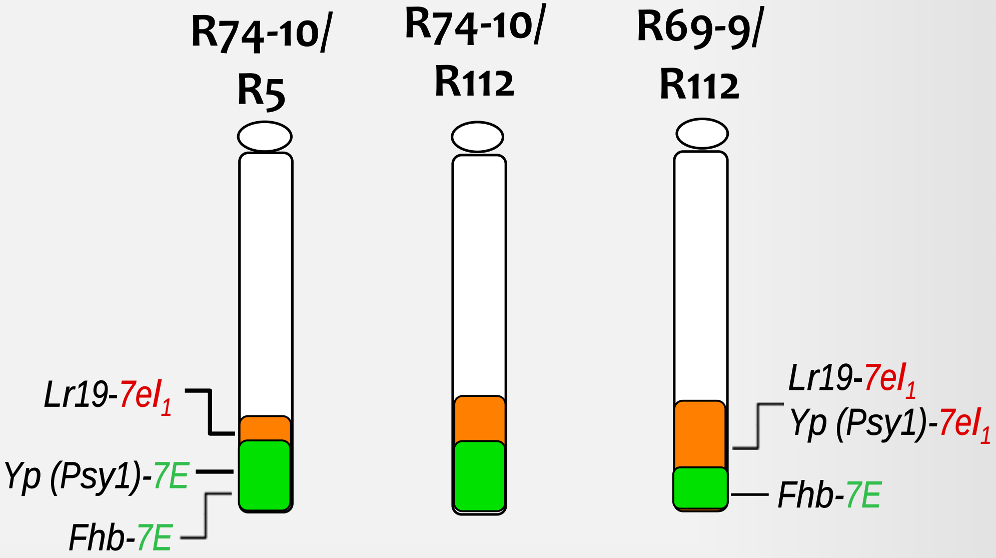
GNS - Grain No/Spike
GNSP - Grain No/Spikelet
GYS - Grain Yield/Spike

- The 2 seasons showed the **R193** genotype (R112+7el2) to perform **better** (HOM+ ≃ HOM-) under more **favorable** (non moisture and high-temperature stress) **conditions** (**2019**)
- By contrast, the **R216** recombinant (R5+7el2) showed **better** adaptive ability to **stressful conditions** (**2020**)
- Same differential performance observed for R5 and R112 → **7el1-linked effects, maintained in R193/R216**

2019 - 7el1+7E recombinants



- As a whole → no major negative effects on spike fertility and yield for the three 7el1+7E recombinants tested in 2019
- However, the **best performing** genotypes resulted the R112 derivatives (as for 7el1+7el2 types), particularly **R69-9/R112**
- The **poorest** performance was exhibited by **R74-10/R5,** having the largest 7EL and the smallest 7el1L segment



- R69-9 derivatives (*Yp*-7el1), both **R69-9/R112** and **R69-9/R5** (later obtained), were selected for further trials and use in breeding

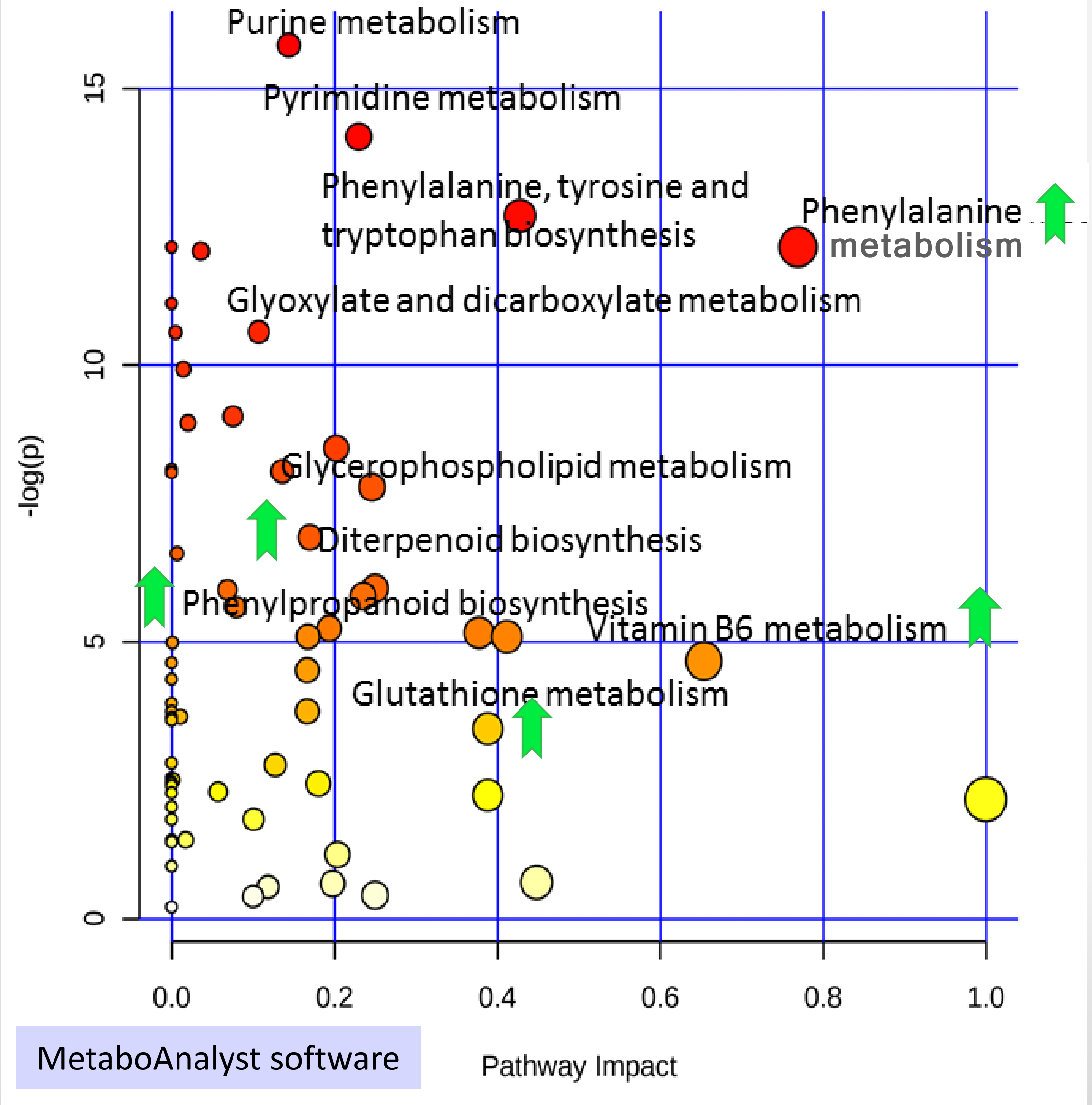
2020 – All recombinants, spaced plants in rows (triplicated, randomized)

	Genotype	Alien segments	Plant traits				Spike traits			
			GY	TILN	GN	TGW	SPN	GNS	GNSP	GYS
7el1+7E	R69-9/R5+	7el$_1$+7E	13.4	5.0 ab	213.5	63.4 ab	19.6 a	45.6 ab	2.3 b	2.9 ab
	R69-9/R112+	7el$_1$+7E	11.3	4.2 ab	191.2	59.1 bc	18.4 ab	50.7 a	2.7 a	3.0 a
7el1+7el2	R216+/IDYT22	7el$_1$+7el$_2$	10.8	5.5 a	208.1	53.0 c	18.5 ab	45.8 ab	2.5 ab	2.5 bc
	R193+/IDYT22	7el$_1$+7el$_2$	10.3	4.9 ab	203.1	51.0 c	17.1 c	46.3 ab	2.7 a	2.5 bc
7el1 only	R5+	7el$_1$	8.8	3.7 ab	149.2	59.6 abc	15.8 d	42.7 bc	2.7 a	2.5 bc
	R112+	7el$_1$	8.3	3.8 ab	157.4	54.2 c	15.8 d	43.8 bc	2.7 a	2.4 c
NO alien	IDYT22	–	10.2	4.6 ab	157.8	64.3 ab	16.6 cd	37.4 c	2.2 b	2.3 c
	Simeto	–	9.2	3.3 b	134.8	66.7 a	17.5 bc	41.4 bc	2.4 b	2.8 ab
	ANOVA *p* value		0.185	0.010 *	0.050	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000

- **No significant yield differences (plant & spike)**
- **Best performing** → 7el1+7E genotypes, but all **recombinants with "nested" segments,** irrespective of the background, had better or, at least, equivalent values for yield-contributing traits compared with those carrying 7el1 segments only and recurrent DW varieties
- MAS breeding is currently underway

The untargeted metabolomics profile (LC-MS) of the rachis tissue of 7E+ (HOM+) vs. 7E– (HOM–) R69-9/R5 NIRLs ---- 2-4 days post inoculation (dpi) with *F. graminearum* (*Fg*)

Metabolomics Pathway Analysis (MetPA) - **4 dpi**



- Several metabolic routes significantly affected in the response to *Fg* inoculation (🔴 > 🟠 > 🟡)
- **Main changes between 7E HOM+ and HOM–** involved **phenylalanine** metabolism, **phenylpropanoid** (**PPN**) and **diterpenoid** biosynthesis, all main routes known to be activated in response to *Fusarium* inoculation
- The complex **PPN pathway** includes production of **LIGNIN** → **precursors up-regulated in 7E HOM+** → **hardening of cell wall** → **physical barrier**
- **PPN is** also a **primary source of ROS scavenging compounds** → **anti-oxidant defense**
- Potent **anti-oxidants** activated **in stressed plants** are also **B6 VITAMERS** → **specifically induced in HOM+ plants**
- **Anti-oxidant activity** by several metabolites of **glutathione (GSH) metabolism**, much **more abundant in HOM+** than HOM- plants
- **Exclusive** presence in DW **Fhb7E+** rachises of **de-epoxydated DON-GSH adduct** → confirmed Wang et al.'s findings in DON-treated seedlings/detached leaves → *Fhb7* encodes a GST (Science 2020)

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