Barley Breeding at ICARDA: from traditional Breeding to genomic selection

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Improving the current winter barley program….

A) Pre-Breeding and Trait Discovery:

- Through the exploration of barley genetic resources (FIGS subsets, landraces and wild relatives from our genebank and/or other sources) and the evaluation of their performances in different stress prone environments we aim to identify germplasm with increased tolerance to abiotic and biotic stress.

- GWAS (multipurpose winter association mapping panel) and bi-parental mapping for abiotic and biotic stress tolerance and quality traits. The panel will assembled using landraces, cultivars with different origin and genotypes from our crossing block. DHs population will be developed form for further studies on trait of interest.

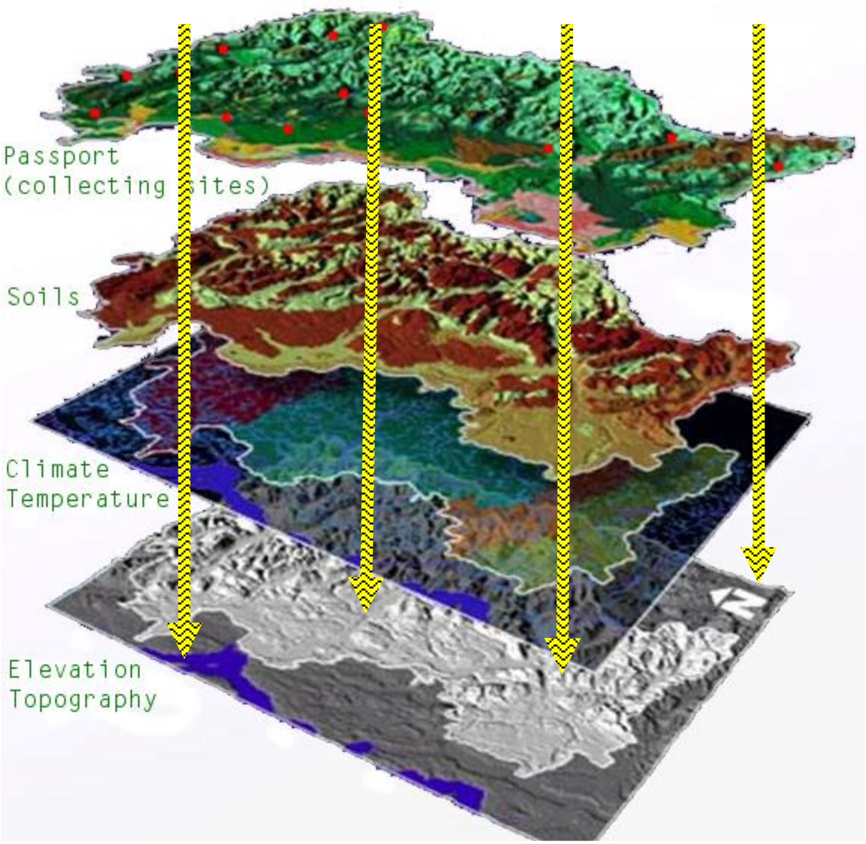
- Use of doubled haploids technology for germplasm development.

- Identifying markers suitable for MAS for germplasm screening for and selection of promising DHs, genotypes that meet requirements for local adaptation and end-use orientation.

B) Introgression of the enhanced abiotic/biotic stresses tolerance and/or quality traits into the improved background of our breeding matrials, for generating germplasm with increased yield, tolerance to abiotic/biotic stress constrains and quality, through systematic hybridization (enclosing winter x spring crosses).

- *Crossing elite lines with selected H.spontaneum and backcross*
- *Top crosses [elite x landraces] x elite*
- *Doubled haploid production/evaluation*
- *Winter x spring crossing for desirable traits*

Link environmental data to collection sites



Choose accessions from that environments that would impose a selection pressure for a given trait.

i.e: for heat tolerance, select material from environments where there is a high selection pressure

Link environmental data to collection sites

FIGS combines agro-ecological information with data on plant traits and characteristics. FIGS datasets identify sets of plant genotypes with a higher probability of containing specific ‘target’ traits.

Sunn Pest– first time in bread wheat

RWA resistance – new genes indicated

Hessian fly - resistance to US bio-type

Powdery mildew – 2 new functional genes

Salinity – 20% of salinity set showed resistance compared to only 3% of core set

Drought – FIGS performed better than core

FIGS searches can be done for you by the ICARDA FIGS team on request.

Developed by ICARDA, GRDC and Vavilov Institute.

FIGS: winter barley subsets

Winter barley FIGS subsets

Frost tolerance 152 entries
Drought 270 (60 winter/facultative)
Powdery mildew 200 (31 winter/facultative)
Scald 96 (15 winter/facultative)

Stripe rust 64 (13 winter/facultative)
Leaf Rust 110 (30 winter/facultative)
Net bloch 77 (10 winter/facultative)

- Test the subset for frost tolerance in different frost prone environments i.e. Sivas in Turkey, (only for assessing frost damage recovery). Phenotyping for frost tolerance and for agronomic traits/disease. Genotypes with enhanced frost tolerance identified can be used in our crossing programs to increase frost tolerance.

- Test the 60 w/f from the drought subset in cold/drought locations across the region.

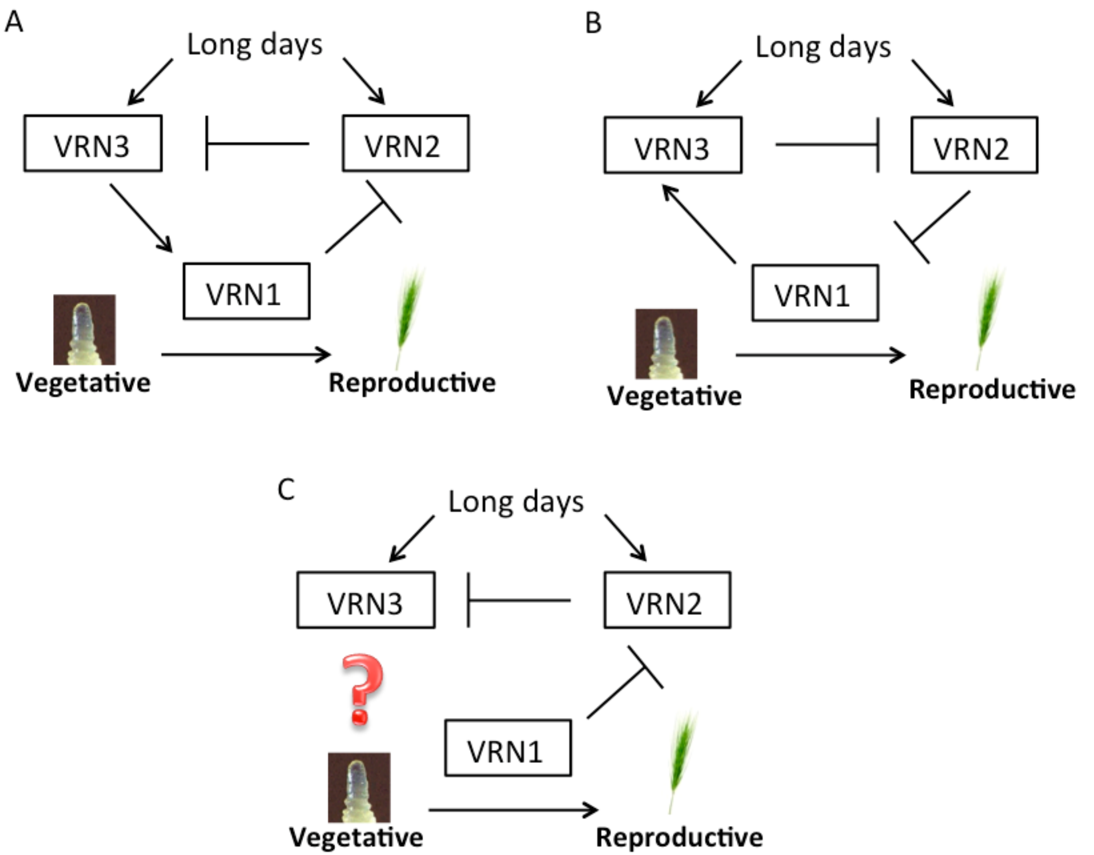
- Assembly a winter/facultative nursery for multiple disease, using winter/facultative genotypes form the above listed subsets. The nurseries can be tested in different locations: Kastamonu in Turkey, Annoucer in Morocco, Kfardan in Lebanon etc. and finally distributed to NARS partners.

Introgression of desirable traits:

Introgression of the enhanced abiotic/biotic stresses tolerance and/or quality traits into the improved background of our breeding materials, for generating germplasm with increased yield, tolerance to abiotic/biotic stress constrains and quality, through systematic hybridization (enclosing winter x spring crosses).

- Crossing elite lines with selected H.spontaneum and backcross
- Top crosses [elite x landraces] x elite
- Doubled haploid production/evaluation
- Winter x spring crossing for desirable traits

Combining MAS with DHs: breeding for quality



Targeted cro
DHs producti
i.e. winter eli
spring maltir
with/high β-
(with disease
resistance).

DHs production of genotypes
of interest
mal).
so enclosed in
n
e rejected.

Vernalization: V
primer availabl
marker. No dia

nts identified),
2 presence/absence

Malting quality
to select differen
malting extract).

PCR assay is available
ated with increased

β-glucans: Bree
(Xue et al. 1997).
amylose/amylop
deletion in the p

starch on β-glucan
er the
types is due to a
of β-glucans content

(Chutimanitsakun et al. 2013). Double purpose marker!

Diseases: powdery mildew *(mlo),* leaf rust *(Lr34),* stripe rust (Yr18; Yr36 temperature dependent)…..

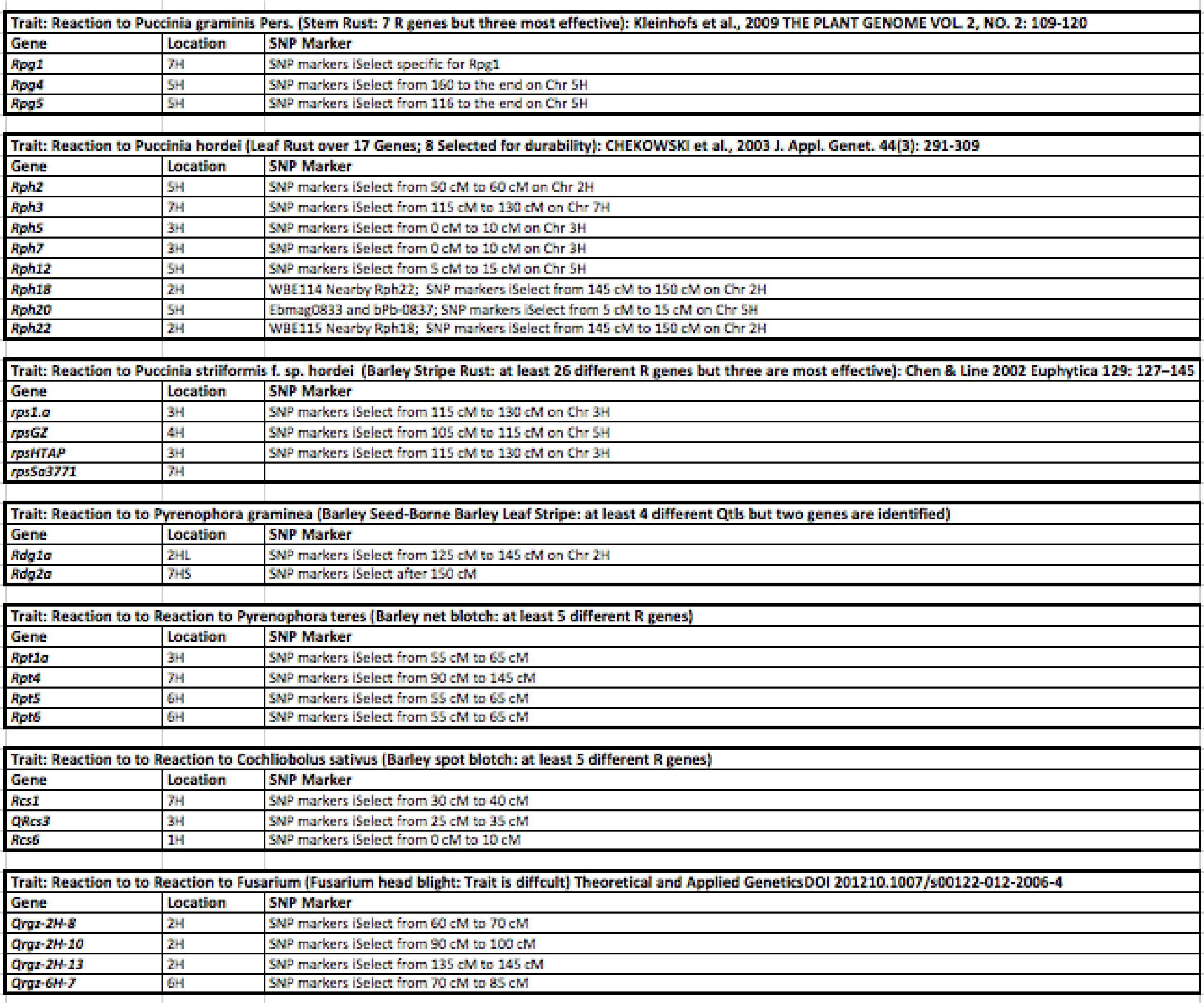
Quality food and malting

Instrument	Analysis	Status
Sortimat	Kernel Size	operational
GrainScan	Length / width / Area / perimeter	operational
Granolyzer NIR	Protein / Moisture	operational
Granomat NIR	Moisture / Sp weight	operational
Foss NIR	Protein / Moisture / β-gluc	operational
iCAP	Micronutrient (Fe, Zn, Mg, Mn…)	operational
Micromalting system	Malt quality	operational
Sen++	Malting	operational

- Phenotyping AM panels, DHs (Zn and Fe), CB and advanced breeding materials
- GWAS/QTL analysis, molecular marker validation i.e. Fe & Zn
- Selection of advanced breeding materials for quality traits
- Combining MAS with malting quality analysis. MAS for the main important MQ parameters, grain physical parameters and micromalting. This will reduce the number of genotypes to be tested and consequently the cost…
- Materials with enhanced quality traits can be used for crossings

MAS: validated markers for disease resistance

ICARDA
Science for resilient livelihoods in dry areas



Trait: Reaction to Puccinia graminis Pers. (Stem Rust: 7 R genes but three most effective): Kleinhofs et al., 2009 THE PLANT GENOME VOL. 2, NO. 2: 109-120

Gene	Location	SNP Marker
Rpg1	7H	SNP markers iSelect specific for Rpg1
Rpg4	5H	SNP markers iSelect from 160 to the end on Chr 5H
Rpg5	5H	SNP markers iSelect from 116 to the end on Chr 5H

Trait: Reaction to Puccinia hordei (Leaf Rust over 17 Genes; 8 Selected for durability): CHEKOWSKI et al., 2003 J. Appl. Genet. 44(3): 291-309

Gene	Location	SNP Marker
Rph2	5H	SNP markers iSelect from 50 cM to 60 cM on Chr 2H
Rph3	7H	SNP markers iSelect from 115 cM to 130 cM on Chr 7H
Rph5	3H	SNP markers iSelect from 0 cM to 10 cM on Chr 3H
Rph7	3H	SNP markers iSelect from 0 cM to 10 cM on Chr 3H
Rph12	5H	SNP markers iSelect from 5 cM to 15 cM on Chr 5H
Rph18	2H	WBE114 Nearby Rph22; SNP markers iSelect from 145 cM to 150 cM on Chr 2H
Rph20	5H	Ebmag0833 and bPb-0837; SNP markers iSelect from 5 cM to 15 cM on Chr 5H
Rph22	2H	WBE115 Nearby Rph18; SNP markers iSelect from 145 cM to 150 cM on Chr 2H

Trait: Reaction to Puccinia striiformis f. sp. hordei (Barley Stripe Rust: at least 26 different R genes but three are most effective): Chen & Line 2002 Euphytica 129: 127–145

Gene	Location	SNP Marker
rps1.a	3H	SNP markers iSelect from 115 cM to 130 cM on Chr 3H
rpsGZ	4H	SNP markers iSelect from 105 cM to 115 cM on Chr 5H
rpsHTAP	3H	SNP markers iSelect from 115 cM to 130 cM on Chr 3H
rps5a3771	7H	

Trait: Reaction to to Pyrenophora graminea (Barley Seed-Borne Barley Leaf Stripe: at least 4 different Qtls but two genes are identified)

Gene	Location	SNP Marker
Rdg1a	2HL	SNP markers iSelect from 125 cM to 145 cM on Chr 2H
Rdg2a	7HS	SNP markers iSelect after 150 cM

Trait: Reaction to to Reaction to Pyrenophora teres (Barley net blotch: at least 5 different R genes)

Gene	Location	SNP Marker
Rpt1a	3H	SNP markers iSelect from 55 cM to 65 cM
Rpt4	7H	SNP markers iSelect from 90 cM to 145 cM
Rpt5	6H	SNP markers iSelect from 55 cM to 65 cM
Rpt6	6H	SNP markers iSelect from 55 cM to 65 cM

Trait: Reaction to to Reaction to Cochliobolus sativus (Barley spot blotch: at least 5 different R genes)

Gene	Location	SNP Marker
Rcs1	7H	SNP markers iSelect from 30 cM to 40 cM
QRcs3	3H	SNP markers iSelect from 25 cM to 35 cM
Rcs6	1H	SNP markers iSelect from 0 cM to 10 cM

Trait: Reaction to to Reaction to Fusarium (Fusarium head blight: Trait is diffcult) Theoretical and Applied GeneticsDOI 201210.1007/s00122-012-2006-4

Gene	Location	SNP Marker
Qrgz-2H-8	2H	SNP markers iSelect from 60 cM to 70 cM
Qrgz-2H-10	2H	SNP markers iSelect from 90 cM to 100 cM
Qrgz-2H-13	2H	SNP markers iSelect from 135 cM to 145 cM
Qrgz-6H-7	6H	SNP markers iSelect from 70 cM to 85 cM

Genome wide association analysis (GWAS) in brief:

- Method to map QTL that takes advantages of the historic linkage disequilibrium to link phenotypes and to genotypes.
- Allows to map QTL with an higher resolution than other mapping methods such as bi-parental populations. Furthermore the assembly a of bi-parental population require more time than an association panel.
- Could be used to identify the candite gene(s) underlying the trait of interest. Enhancing the efficency of gene discovery and facilitating MAS (Marker Assisted Selection) in plant breeding (Gupta et al. 2005: Moose and Mumm 2008).
- GWAS (Genome Wide Association Studies) and Linkage Disequilibrium (LD) are a promising tool for detection and fine mapping of QTL underlying complex agronomics traits such as agronomical traits (row type, heading date, plant height, TKW, starch content, protein content, and also drought and cold stress and aluminum tolerance, disease resistence etc.).
- Could be also used to identify genes underlying qualitative traits.

GWAS in brief:

1. Association Panel: a collection of different genotypes representing a wide germplasm base. This provide the intrinsic nature of exploiting historical recombination events.

2. High throughput genotyping such as Illumina iSelect 9K & 50K or DaRTSeq

3. Phenotypic data fot the trait of interest.

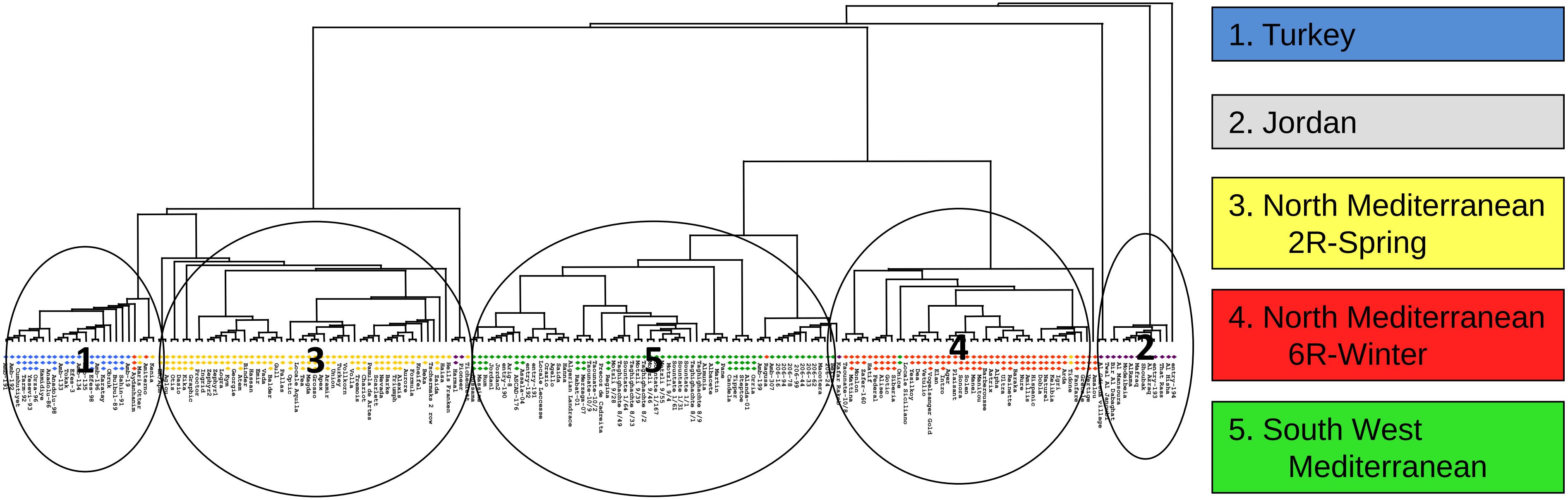
4. Knowledge of population structure (Structure or a simple PCO analysis).

5. Mixed models to controls the effects of population structure and to avoid the detection of spurious associations and false negative discovery.

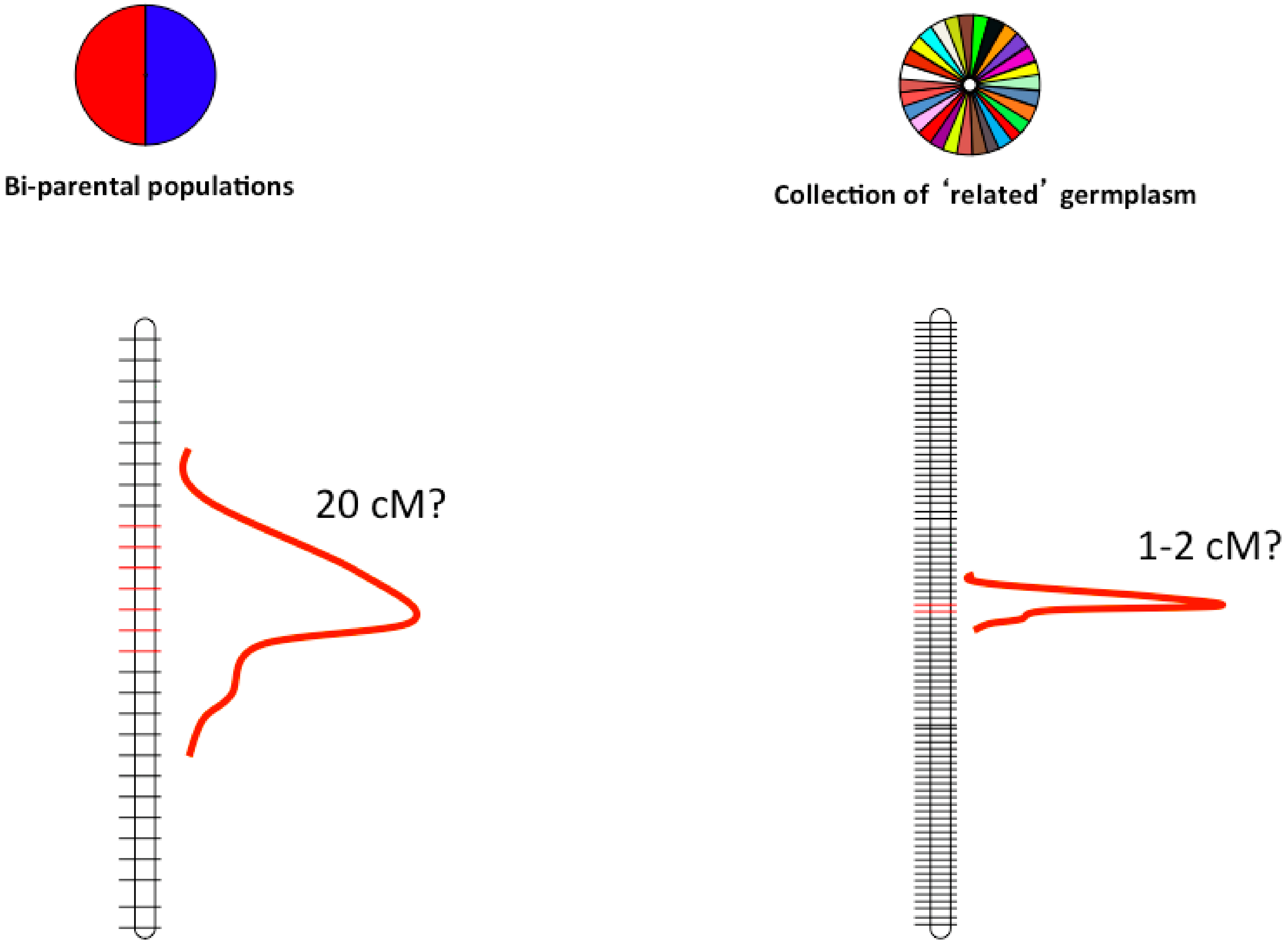
GWAS in brief:

WHAT IS POPULATION STRUCTURE OR POPULATION STRATIFICATION:
Refers to the inclusion of individuals isolated from sub-populations in the association Panel. Individuals from from a sub-population are, on average, more closely related to each other than to other individuals as a whole (Kadri et al 2014).

<u>Population Structure for DBG (Diverse Barley Germplasm)</u>: *185 entries comprising landraces, old and modern cultivars representing the barley gene pool of Mediterranean basin.*



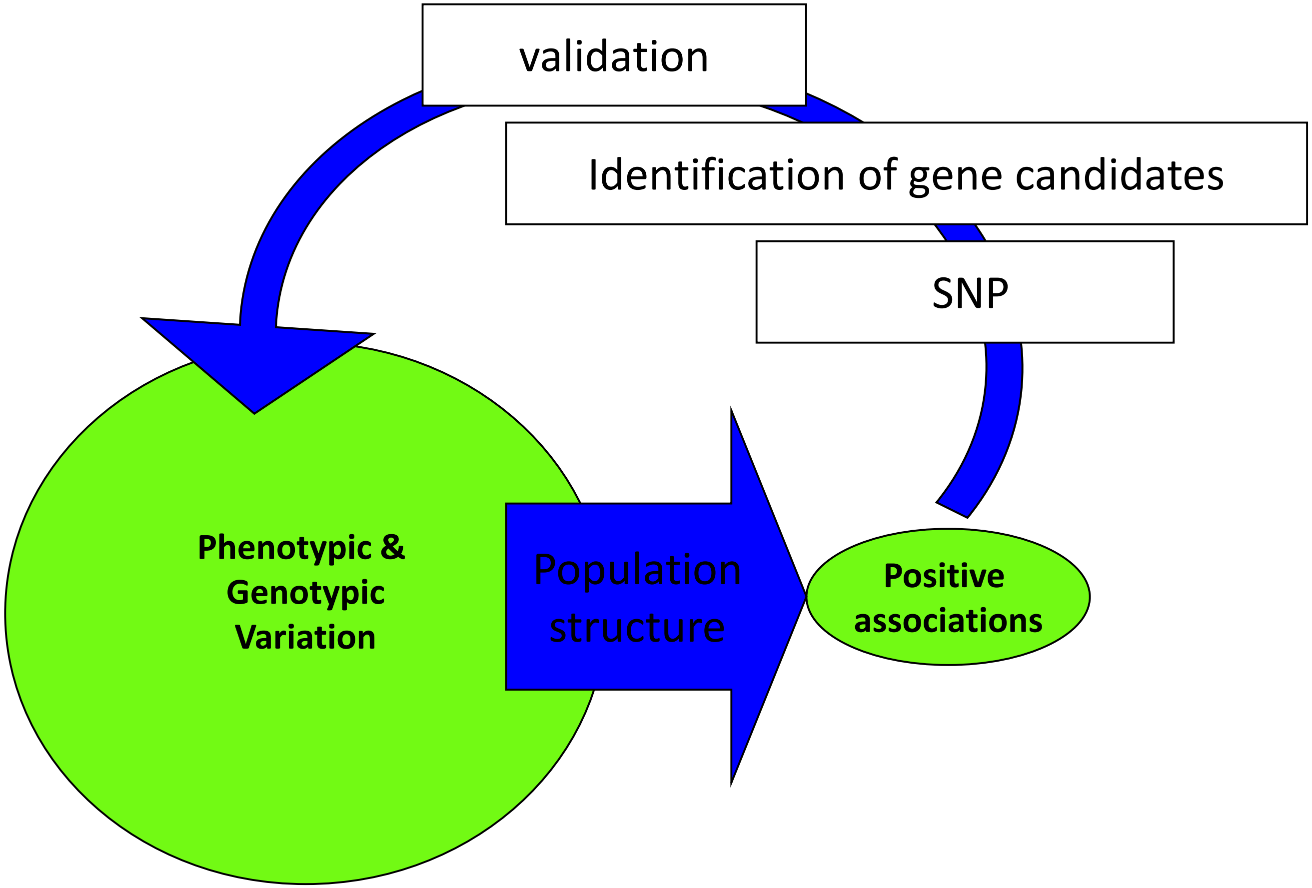
GWAS in brief:



Bi-parental populations

Collection of ‘related’ germplasm

GWAS in brief:


validation

Identification of gene candidates

SNP

Phenotypic & Genotypic Variation

Population structure

Positive associations

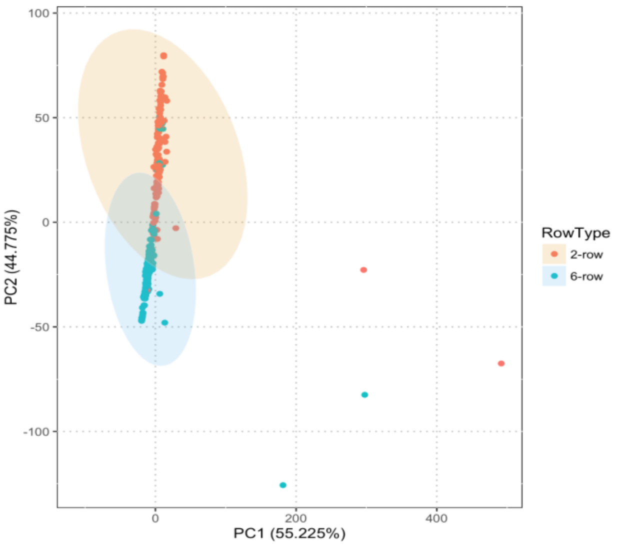
Pre-breeding and trait discovery: Li-AM

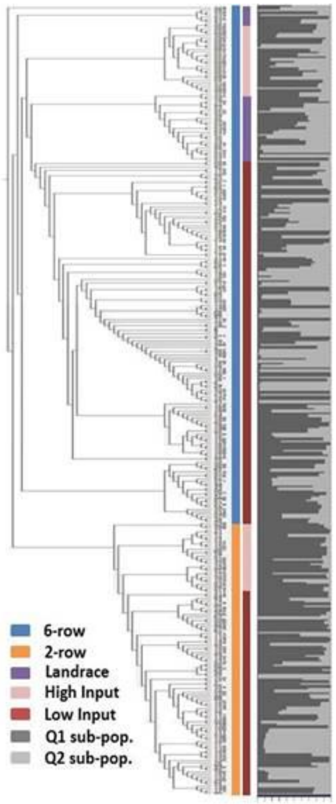
- Li-AM: a collection of 336 genotypes (AM panel) from ICARDA germplasm, geographically diverse and consisting of released cultivars, advanced breeding lines, landraces and differentials .

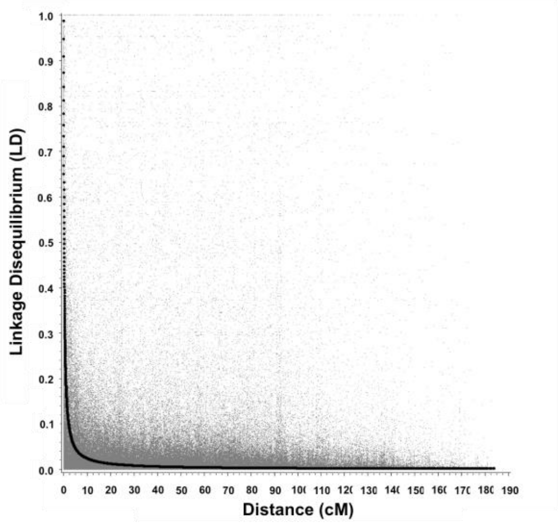
- Genotyped with iSelect 9K Chip (Illumina), after removing markers with MAF < 5% and missing data > 10% a set of 6.5K SNPs were used for GWAS analysis.

- Li-AM is going to be tested for the second year for agronomics traits, net bloch (SFNB and NFNB), powdery mildew, heat and drought, salinity and b-glucans, straw and grain quality and microelements (Fe and Zn).

- Performing GWAS with the aim of identifying markers linked to traits of interest and use them for MAS after validation







Gyawali et al. 2015

Pre-breeding and trait discovery: Hi-AM

- 280 genotypes enclosing released cultivars, advanced lines and local landraces, collected from diverse sources: ICARDA breeding program, North America, South America, Europe and Australia.

- Genotyped with DaRT-Seq, after removing markers with MAF < 5% and missing data > 10%, 25K PAVs and 6K SNPs suitable for GWAS analysis.

- GWAS for yellow rust, root seminal traits and net bloch (SFNB and NFNB), agronomic traits, straw and grain quality, abiotic stress, nutrient content and for quality traits and malting traits

- Identifying markers linked to traits of interest and use them for MAS after validation

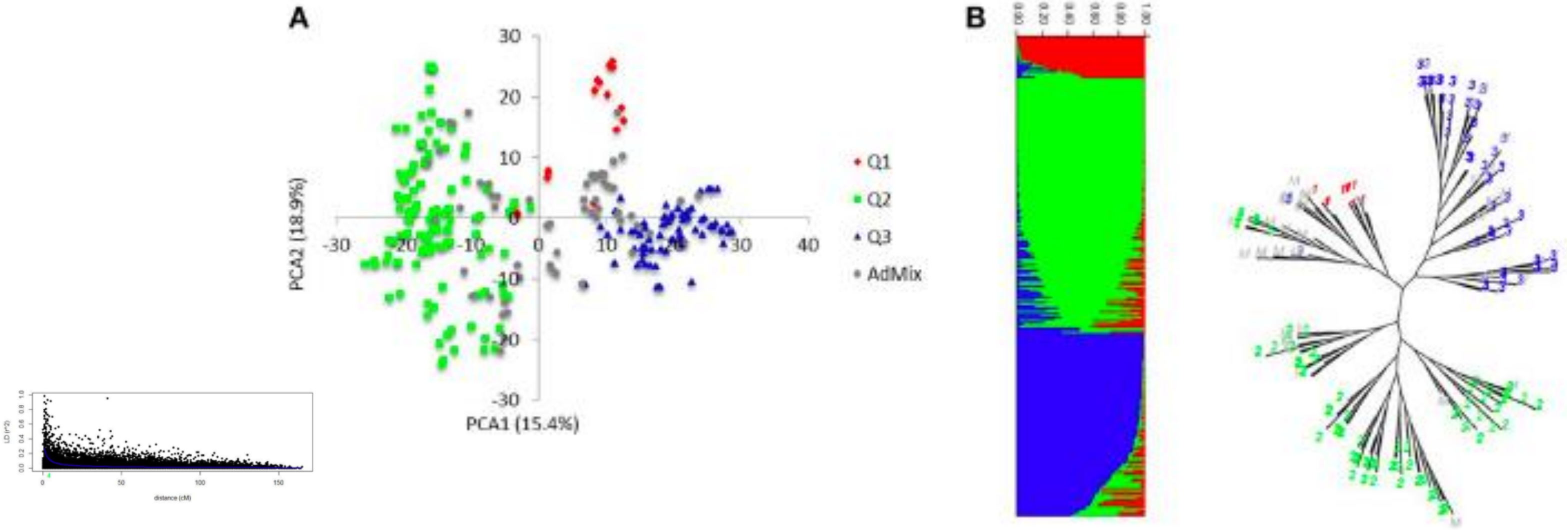


FIGURE 1 | Population structure and linkage disequilibrium. **(A)** Principal component analysis of the HI-AM panel. **(B)** The proportion of the genome of each individual originating from each inferred sub-population, a total of 3 and, each color represent a single sub-population.

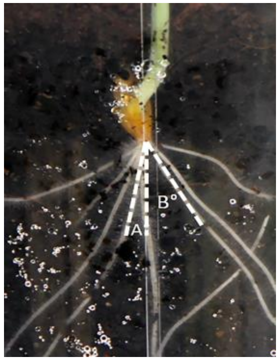
Pre-breeding and trait discovery: Seminal root angle

- Root traits expressed at early plant developmental stages, such as seminal root angle and root number, are associated with timing and extent of water extraction at different depths.

- In wheat, rice and sorghum narrow root angle and high root number (in seedlings) have been identified as precursor for deeper rooting and greater branching at deep. (Robinson et al. 2016)

GWAS for SRA using both AM panels:

- Identifying marker trait associations for SRA, marker validation for MAS
- Dry-down test on selected genotypes
- Correlation with GY
- Multi-location trials (Hi-AM and Li-AM)
- Finding correlation between above ground traits (agronomic traits and phenology, water use efficiency, early vigor, stay green, rate of senescence from anthesis and NDVI) and root architecture traits
- Determine how those traits are related to one another and identifying the best combination of traits for different target environments.

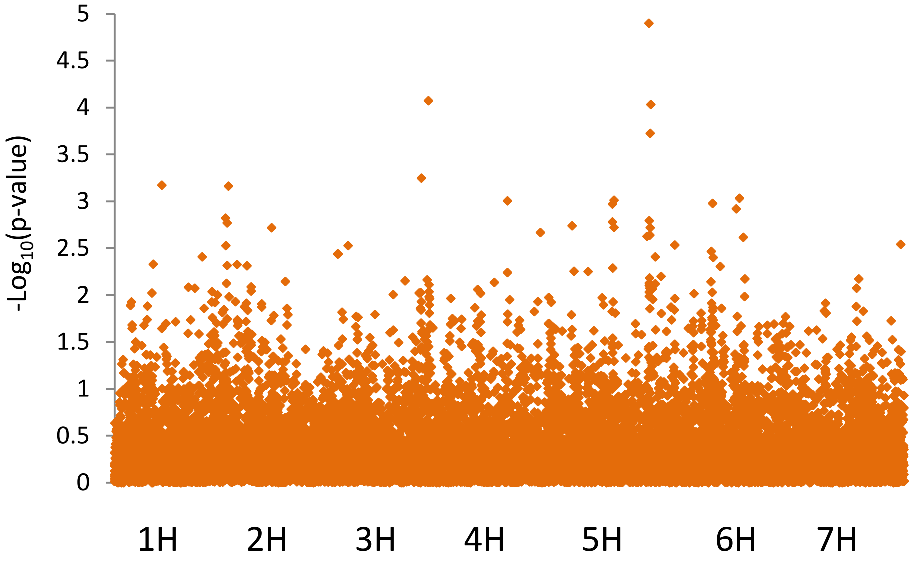


(Richard et al. 2015)

Pre-breeding and trait discovery: Seminal root angle

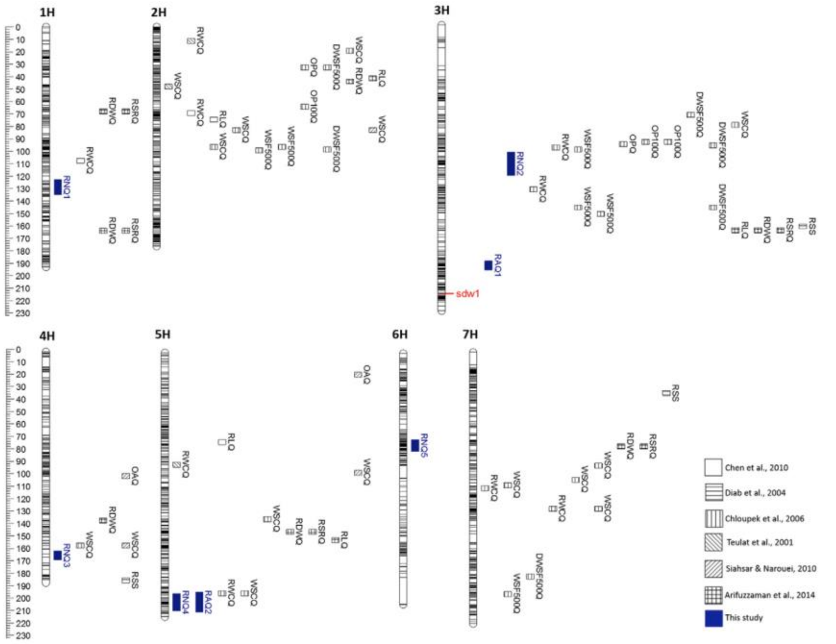
Marker	Chr	cM	-Log10 (P-Val)	MarkerR2	Allele effect	MAF
DaRT793	1H	57.29	3.17	4.65%	-7.4598	22.00%
DaRT1904	2H	9.21	3.16	4.58%	-6.7247	31.50%
DaRT5126	3H	62.68	3.24	4.73%	6.9127	28.40%
DaRT5243	3H	76.35	4.07	6.45%	-9.789	27.76%
DaRT6559	4H	52.34	3.02	4.46%	-5.9745	42.50%
DaRT8340	5H	111.77	3.01	4.36%	-8.4606	47.33%
DaRT8924	5H	152.36	4.90	8.25%	7.19316	40.85%
DaRT8945	5H	152.47	3.72	5.67%	7.19316	24.58%
DaRT8954	5H	152.47	4.03	6.28%	-7.5175	37.20%
DaRT10433	6H	86.26	3.03	4.52%	-6.4423	26.61%

Min:17.00979906 Max: 70.40213273 Avg: 46.21455908 h^2 = 45.8 %



10 marker trait association corresponding to 8 QTL located on chromosomes 1H, 2H, 3H (2), 4H, 5H (2) and 6H.

Some of the QTL are coincident with already reported QTL for12 drought tolerance related traits and with some detected in a DHs population by Robinson et al. 2015. The QTL on chr 4H has never been reported before.

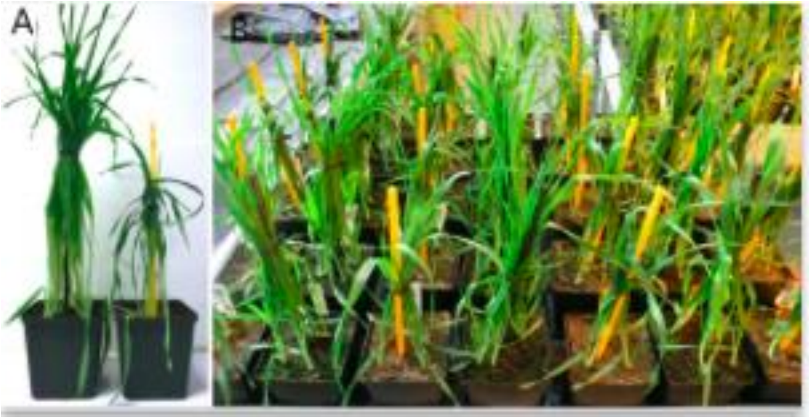


RAQ, root angle; RNQ, root number; RLQ, root length; RDWQ, root dry weight; RSRQ, root to shoot ratio; RSSQ, root system size; RWCQ, relative water content; DWSC100Q, accumulation water-soluble carbohydrate at 100% RWC; OPQ, osmotic potential; OP100Q, osmotic potential full turgor; WSCQ, water-soluble carbohydrate; WSC100Q, WSC full turgor; OAQ, osmotic adjustment..

Pre-breeding and trait discovery: Seminal root angle

RSA in adult plant

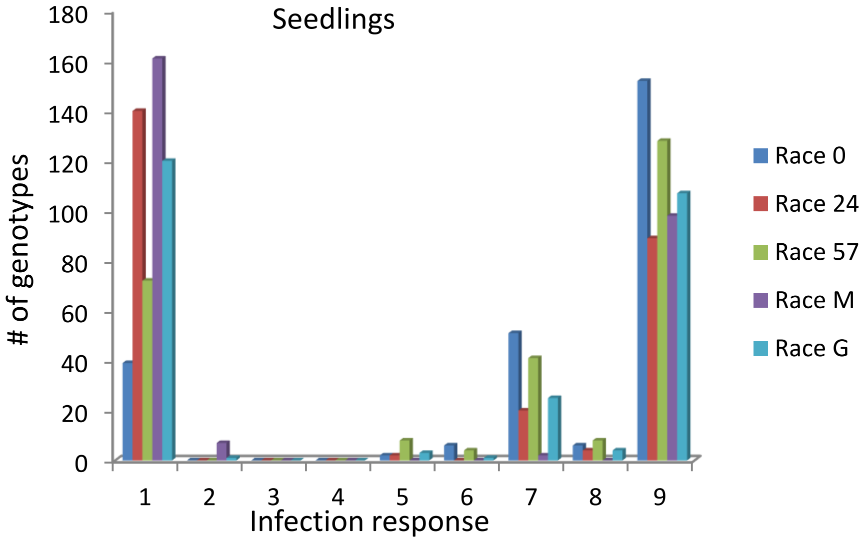
Dry-down

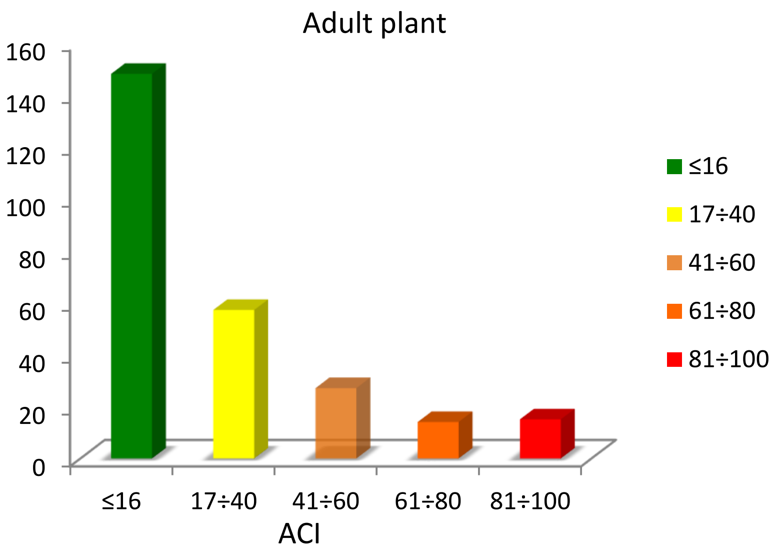


Pre-breeding and trait discovery: stripe rust (India)

The Hi-AM was screened in for both seedling and adult plant resistance to barley stripe rust pathogen (*Puccinia striitiformis* f. sp. *hordei* PSH) in India during cropping season 2013/14, 2014/15 and 2015/16.

- Seedling resistance test were undertaken by inoculating the Hi-AM panel with the five prevalent races in India [0 (5S0), 24 (0S0-1), 57 (0S0), M (1S0) and G (4S0)
- Screening for adult plant resistance, in the field, were performed in two different location Durganpura (Rajasthan) and Karnal (Haryana) under artificial stripe rust infection using a mixture of the five races.





R
- 0 = no visible signs or symptoms (0)
- 1 = necrotic and/or chlorotic flecks; no sporulation (;-)
- 2= necrotic and/or chlorotic blotches or stripes; no sporulation (1)
- 3 = necrotic and/or chlorotic blotches or stripes; trace sporulation (2-)
- 4 = necrotic and/or chlorotic blotches or stripes; light sporulation (2)
- 5 = necrotic and/or chlorotic blotches or stripes; intermediate sporulation (2+ or 22+)

S
- 6 = necrotic and/or chlorotic blotches or stripes; moderate sporulation (3-)
- 7 = necrotic and/or chlorotic blotches or stripes; abundant sporulation (3)
- 8 = chlorosis behind sporulating area; abundant sporulation (33+)
- 9 = no necrosis or chlorosis; abundant sporulation (3+)

R=0.2; MR=0.4; M=0.6; MS=0.8; S=1

20MS➔ 20 x 0.8= 16

Spring barley: GWAS yellow rust results summary

Race 0

Pos (cM)	Gene Identifier	Description	Know co-segregating loci
52.90	MLOC_8615.2	Glucan endo-1,3-beta-glucosidase 4	11_10796 [Toojinda et al. 2000 (APS); Vales et al. 2005 (APS); Gutierrez et al. 2015 (APS)]
51.63	-	-	SCRI_RS_154973 [Rao et al. 2007 (APS)]
61.12	AK356118	Glucan endo-1,3-beta-glucosidase 4	-
151.98	MLOC_6270.1	NBS-LRR disease resistance protein family-1	SCRI_RS_2824 [Verhoven et al. 2011 (APS); Gutierrez et al. 2015 (APS)]
113.24	MLOC_67477.1	Disease resistance protein (CC-NBS-LRR class) family	-
118.77	AK370472	Disease resistance protein	

Race 24

Chr	Pos (cM)	Gene Identifier	Description	Know co-segregating loci
1H	28.88	MLOC_74415.1	Lr21	-
3H	7.01	MLOC_62179.1	NBS-LRR disease resistance protein, putative	-
3H	14.94	MLOC_75090.1	Endo-1,4-beta-xylanase check	-
3H	36.98	MLOC_56904.1	NBS-LRR disease resistance protein homologue	-
3H	42.46	-	-	SCRI_RS_154973 [Rao et al. 2007(APS)]
4H	50.99	-	-	11_20853 [Vales et al. 2005 (APS); Gutierrez et al. 2015 (APS); Klos et al., 2016 (SDL)]
4H	111.33	AK365216	Disease resistance-responsive (dirigent-like protein) family protein	12_31138 [Verhoven et al. 2011 (APS); Gutierrez et al. 2015 (APS)]
5H	3.02	MLOC_67608.3	NBS-LRR disease resistance protein, putative	-
5H	151.88	MLOC_6270.1	NBS-LRR disease resistance protein family-1	SCRI_RS_2824 [Verhoven et al. 2011 (APS); Gutierrez et al. 2015 (APS)]
7H	24.06	MLOC_67182.3	Cc-nbs-lrr resistance protein	-

Race 57

Chr	Pos (cM)	Gene Identifier	Description	Know co-segregating loci
2H	94.55	-	-	3259480|F|0 [Dracatos et al. 2015 (SDL)
3H	45.22	-	-	SCRI_RS_154973 [Rao et al. 2007 (APS)]
3H	59.63	MLOC_51359.1	NBS-LRR-like protein	-
7H	15.37	MLOC_5217.3	Disease resistance protein (CC-NBS-LRR)	-

Spring barley: GWAS yellow rust results summary

Race G				-
Chr	**Pos (cM)**	**Gene Identifier**	**Description**	**Know co-segregating loci**
2H	8.85	MLOC_73747.1	Cc-nbs-lrr resistance protein	-
2H	146.72	MLOC_58526.2	Disease resistance protein (CC-NBS-LRR)	-
4H	68.98	MLOC_74055.1	NAC domain protein	-
4H	110.20	AK365216	Disease resistance-responsive (dirigent-like protein) family protein	12_31138 [Verhoven et al. 2011 (APS) Gutierrez et al. 2015(APS)]
5H	129.65	AK356729	Glucan endo-1,3-beta-glucosidase 3	11_11532 [Valdes et al. 2005 (APS); Gutierrez et al. 2015 (APS)]
6H	53.75	MLOC_13229.1	Disease Resistance Protein	-
7H	97.10	MLOC_16158.3	NB-ARC domain-containing disease resistance protein	-
Race M				-
Chr	**Pos (cM)**	**Gene Identifier**	**Description**	**Know co-segregating loci**
1H	64.02	MLOC_59979.1	NBS-LRR disease resistance protein homologue	-
3H	98.23	-	-	11_21212 [Vales et al. 2005 (APS); Gutierrez et al. 2015 (APS)]
4H	51.42	-	-	11_20853 [Vales et al. 2005 (APS); Gutierrez et al. 2015 (APS); klos et al. 2016 (SDL)]
5H	120.35	MLOC_30580.2	NBS-LRR disease resistance protein	-
5H	133.69	MLOC_10360.2	NBS-LRR disease resistance protein, putative	11_11532 [Vales et al. 2005 (APS); Gutierrez et al. 2015 (APS)]
7H	132.65	MLOC_38424.1	NBS-LRR disease resistance protein	11_10843 [Thomas et al. 1995 (APS); Gutierrez et al. 2015 (APS); Dracatos et al. 2015(SDL)]

Pre-breeding and trait discovery: YR adult plant resistance

a) Adult plant stage

Durgapura 2013

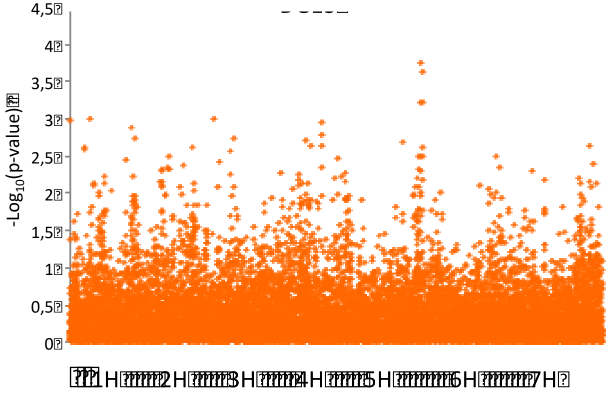
QTL	Chr.	Pos (cM)	Gene Identifier	Description	Know co-segregating loci
*APS_Dg13_1**	1H	40.5	MLOC_11791.2	Disease Resistance Protein	12_30817 [Verhoven et al. 2011(APS)]
APS_Dg13_3	5H	137.08	MLOC_63574.2	Glucan endo-1,3-beta-glucosidase 5	-

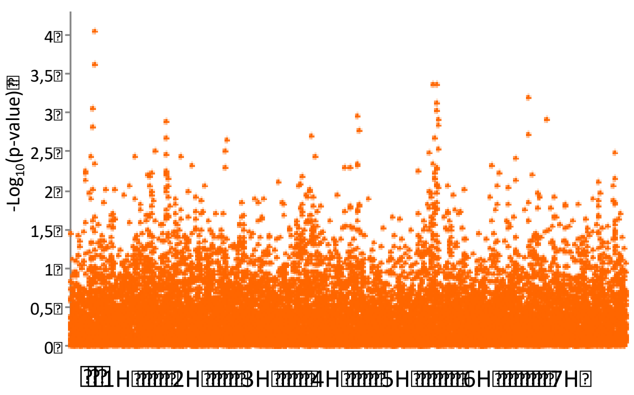
Durgapura 2014

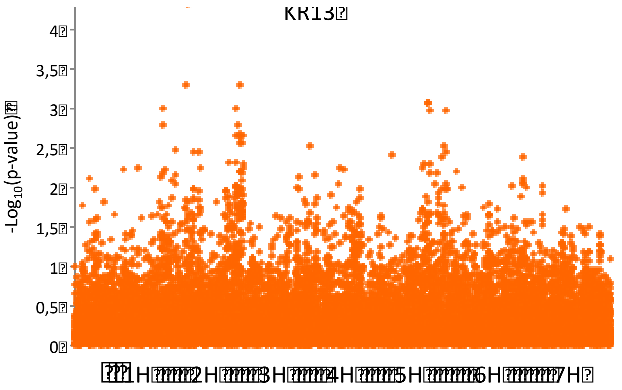
QTL	Chr	Pos (cM)	Gene Identifier	Description	Know co-segregating loci
*APS_Dg14_1**	1H	47.52	MLOC_4500.2	NBS-LRR disease resistance protein homologue	-
APS_Dg14_2	2H	8.85	MLOC_78849.2	disease resistance protein (TIR-NBS-LRR class)	-
APS_Dg14_5	5H	137.08	MLOC_63574.2	Glucan endo-1,3-beta-glucosidase 5	-
APS_Dg14_6	6H	119.12	MLOC_43055.1	Disease Resistance Protein	-

Karnal 2014

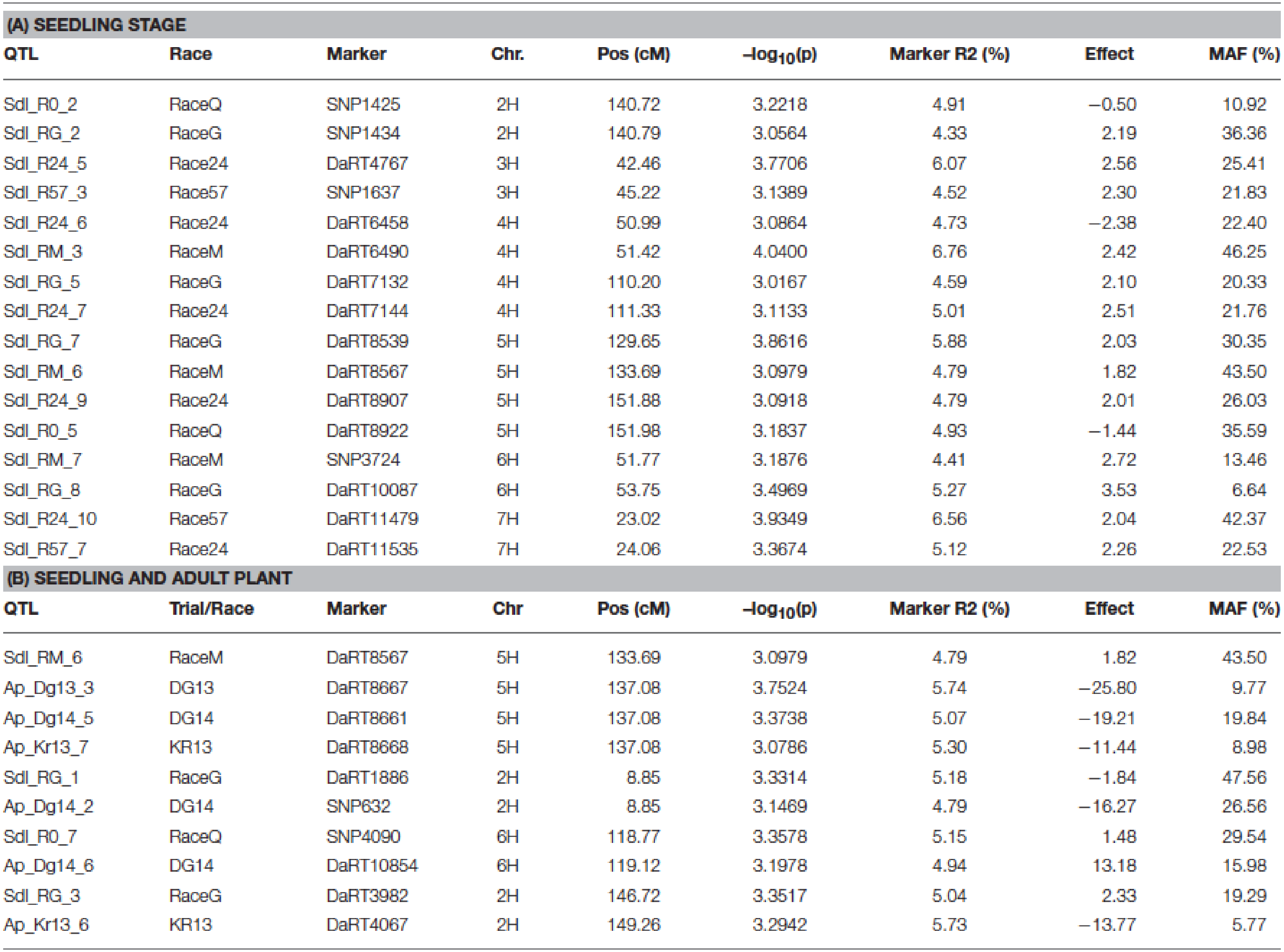
QTL	Chr	Pos (cM)	Gene Identifier	Description	Know co-segregating loci
*APS_Kr14_1**	1H	103.82	MLOC_54911.1		3263737|F|0 [Dracatos et al. 2015 (SDL)]
*APS_Kr14_4**	2H	64.83	MLOC_34376.1	Endo-1,4-b-D-glucanase	3258146|F|0 [Dracatos et al. 2015 (SDL)]
APS_Kr14_6	2H	149.26	MLOC_19010.2	TIR-NBS-LRR class disease resistance protein	-
*APS_Kr14_7**	5H	137.08	MLOC_63574.2	Glucan endo-1,3-beta-glucosidase 5	-
*APS_Kr14_8**	5H	159.51	MLOC_58845.1	NBS-LRR disease resistance protein family-1	-





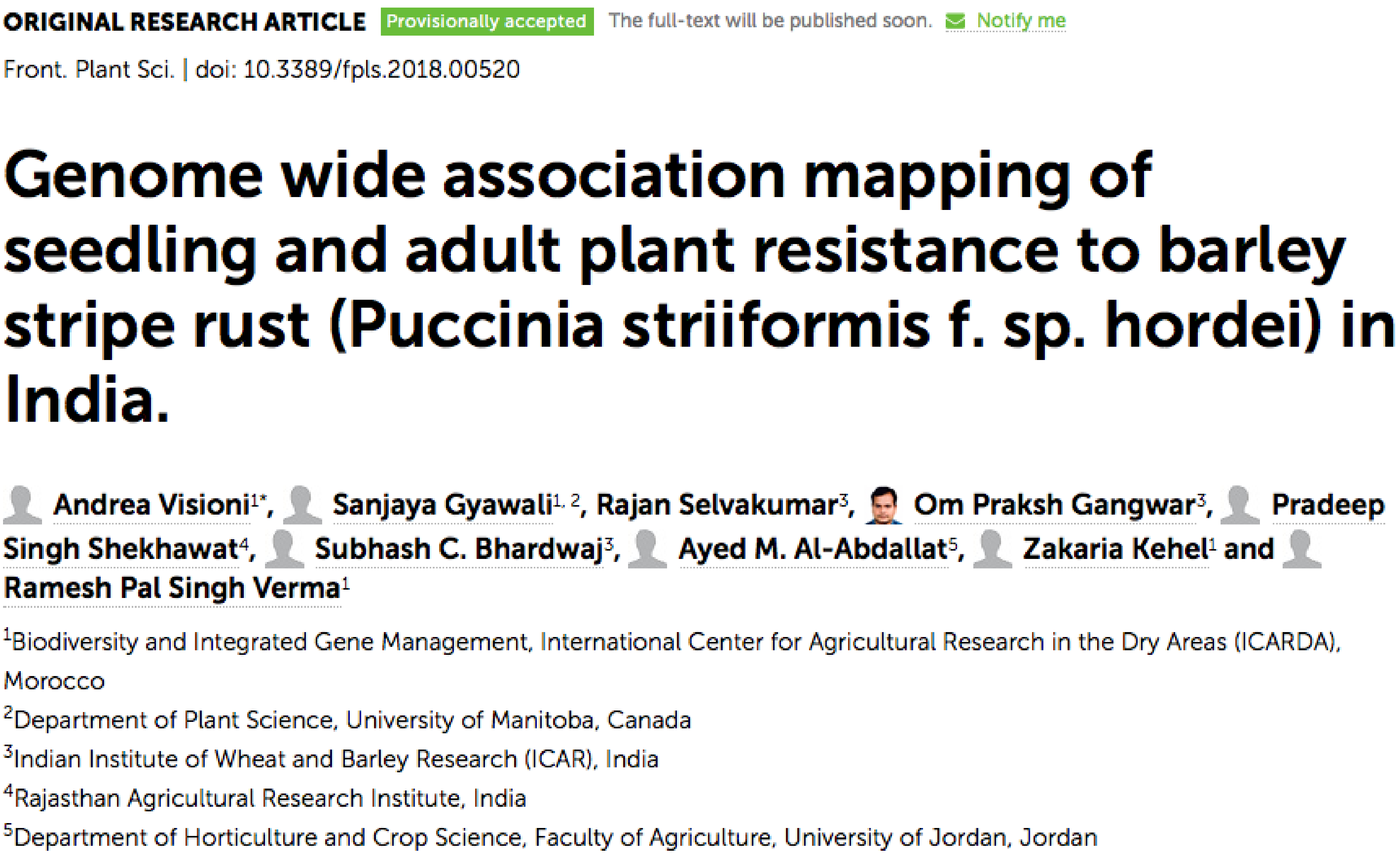


Spring barley: GWAS yellow rust results summary



(A) SEEDLING STAGE								
QTL	**Race**	**Marker**	**Chr.**	**Pos (cM)**	**$-\log_{10}(p)$**	**Marker R2 (%)**	**Effect**	**MAF (%)**
Sdl_R0_2	RaceQ	SNP1425	2H	140.72	3.2218	4.91	−0.50	10.92
Sdl_RG_2	RaceG	SNP1434	2H	140.79	3.0564	4.33	2.19	36.36
Sdl_R24_5	Race24	DaRT4767	3H	42.46	3.7706	6.07	2.56	25.41
Sdl_R57_3	Race57	SNP1637	3H	45.22	3.1389	4.52	2.30	21.83
Sdl_R24_6	Race24	DaRT6458	4H	50.99	3.0864	4.73	−2.38	22.40
Sdl_RM_3	RaceM	DaRT6490	4H	51.42	4.0400	6.76	2.42	46.25
Sdl_RG_5	RaceG	DaRT7132	4H	110.20	3.0167	4.59	2.10	20.33
Sdl_R24_7	Race24	DaRT7144	4H	111.33	3.1133	5.01	2.51	21.76
Sdl_RG_7	RaceG	DaRT8539	5H	129.65	3.8616	5.88	2.03	30.35
Sdl_RM_6	RaceM	DaRT8567	5H	133.69	3.0979	4.79	1.82	43.50
Sdl_R24_9	Race24	DaRT8907	5H	151.88	3.0918	4.79	2.01	26.03
Sdl_R0_5	RaceQ	DaRT8922	5H	151.98	3.1837	4.93	−1.44	35.59
Sdl_RM_7	RaceM	SNP3724	6H	51.77	3.1876	4.41	2.72	13.46
Sdl_RG_8	RaceG	DaRT10087	6H	53.75	3.4969	5.27	3.53	6.64
Sdl_R24_10	Race57	DaRT11479	7H	23.02	3.9349	6.56	2.04	42.37
Sdl_R57_7	Race24	DaRT11535	7H	24.06	3.3674	5.12	2.26	22.53
(B) SEEDLING AND ADULT PLANT								
QTL	**Trial/Race**	**Marker**	**Chr**	**Pos (cM)**	**$-\log_{10}(p)$**	**Marker R2 (%)**	**Effect**	**MAF (%)**
Sdl_RM_6	RaceM	DaRT8567	5H	133.69	3.0979	4.79	1.82	43.50
Ap_Dg13_3	DG13	DaRT8667	5H	137.08	3.7524	5.74	−25.80	9.77
Ap_Dg14_5	DG14	DaRT8661	5H	137.08	3.3738	5.07	−19.21	19.84
Ap_Kr13_7	KR13	DaRT8668	5H	137.08	3.0786	5.30	−11.44	8.98
Sdl_RG_1	RaceG	DaRT1886	2H	8.85	3.3314	5.18	−1.84	47.56
Ap_Dg14_2	DG14	SNP632	2H	8.85	3.1469	4.79	−16.27	26.56
Sdl_R0_7	RaceQ	SNP4090	6H	118.77	3.3578	5.15	1.48	29.54
Ap_Dg14_6	DG14	DaRT10854	6H	119.12	3.1978	4.94	13.18	15.98
Sdl_RG_3	RaceG	DaRT3982	2H	146.72	3.3517	5.04	2.33	19.29
Ap_Kr13_6	KR13	DaRT4067	2H	149.26	3.2942	5.73	−13.77	5.77

Spring barley: GWAS yellow rust results summary



ORIGINAL RESEARCH ARTICLE Provisionally accepted The full-text will be published soon. ✉ Notify me

Front. Plant Sci. | doi: 10.3389/fpls.2018.00520

Genome wide association mapping of seedling and adult plant resistance to barley stripe rust (Puccinia striiformis f. sp. hordei) in India.

Andrea Visioni[1*], **Sanjaya Gyawali**[1, 2], **Rajan Selvakumar**[3], **Om Praksh Gangwar**[3], **Pradeep Singh Shekhawat**[4], **Subhash C. Bhardwaj**[3], **Ayed M. Al-Abdallat**[5], **Zakaria Kehel**[1] and **Ramesh Pal Singh Verma**[1]

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[2] Department of Plant Science, University of Manitoba, Canada
[3] Indian Institute of Wheat and Barley Research (ICAR), India
[4] Rajasthan Agricultural Research Institute, India
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Overview of the ICARDA barley breeding program :

Shuttle breeding Mexico-Morocco, malting barley

Turkey:
Winter Crossing blocks
Winter Breeding materials
Yield trials evaluation
Disease screenings
Frost/Cold and drought

Feed

Lebanon:
Crossing blocks
Breeding materials
Yield trials evaluation
Disease screenings
Heat and drought
Feed, Malt and Food
Off-season

Morocco:
Crossing blocks
Breeding materials
Yield trials evaluation
Disease screenings
Heat and drought
Biofortification
Feed, Malt and Food

India/Nepal:
Breeding materials
Yield trials evaluation
Disease screenings
Malting barley

Ethiopia:
Breeding materials
Yield trials evaluation
Disease screenings
Malting barley

✖ Primary test sites

✖ Secondary test sites

<u>Increased grain yield and biomass</u>

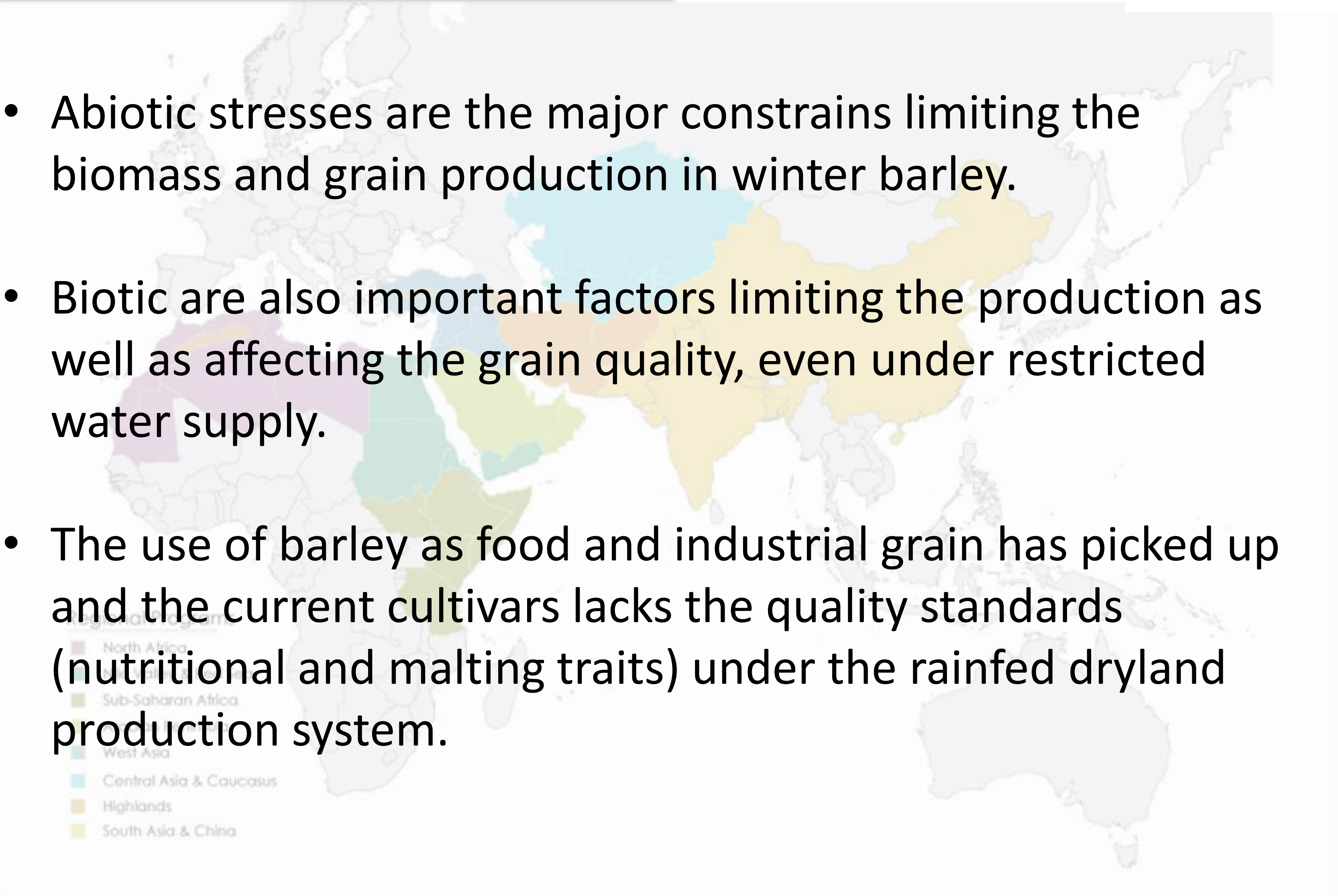
Abiotic stress:
- Drought
- Heat
- Cold/frost
- Salinity

Biotic stress:
- Stripe rust
- Leaf rust
- Stem rust
- Powdery mildew
- Net and spot bloch
- Scald

Quality traits:
- Malting
- B-glucans
- Increased Fe and Zn content

<u>60% animal feed, 30 malting and 10% human feed.</u>

Winter barley: focal points



- Abiotic stresses are the major constrains limiting the biomass and grain production in winter barley.
- Biotic are also important factors limiting the production as well as affecting the grain quality, even under restricted water supply.
- The use of barley as food and industrial grain has picked up and the current cultivars lacks the quality standards (nutritional and malting traits) under the rainfed dryland production system.

Winter barley:

Focal regions and countries:

- Central Asia: Kazakhstan, Uzbekistan, Turkmenistan, Tajikistan, Azerbaijan.
- West Asia: Iran, Turkey, Armenia, Georgia.
- South Asia: Pakistan, Afghanistan, Nepal.
- North Africa: Morocco, Algeria (highlands and mountain areas).


Addressing more than 6 Mha!!!

Objectives:

- Germplasm adapted to both low and high input potential areas
- Increased grain yield
- Increased biomass
- Cold tolerance
- Pre-breeding/trait discovery activities
- Frost tolerance
- Drought and heat stress tolerance
- Salinity (in some focal country)
- Biotic stress tolerance/resistance
- Malting quality (in some focal country)
- Nutrient content.

Winter barley

17Nursery	17 # of Ent.	18Nursery	18 # of Ent.	Location
17WBCB	168	18WBCB	168	Ankara
17SBCB	410	18SBCB	410	Haymana
17Cross	-	18WBF1	160	Haymana
17WBF1	206	18WBF2	206	Haymana
17WBF2	181	18WBF3	181	Haymana
17WBF3	-	18WBF4	-	-
17WBF4-12	226	18WBHR-12	150	Haymana, Kfardan, Lanaceur, Marageh (DARI)
17WBF4	61	18WBHR	31	Haymana, Kfardan, Lanaceur Marageh(DARI)
17WBHR	97	18WBON	224	Hyamana
17WBON	292+32(Iran)	18WBPYT*	176+checks (220)	Haymana
17WBPYT	70	18WBYT**	29	Haymana, Eskesehir, Konia, Kfardan, Lanaceur
17WIBYT	24	C19WIBON-1	24	Seed Increase Terbol
17WBAYT	23	C19WIBON-2	23	Seed Increase Terbol
C19WBIYT	20	C19WBIYT	20	Seed IncreaseTerbol

*Augmented design
** Alpha lattice

Haymana station:

•cold temperate climate
•average temperature of 10 ˚C (minimum temperature can go down to -15 ˚C)
•average long term rainfall of 350 mm (in 2015 we had 800mm)
•early snowfall (snow is covering crops only at early stage)
•Late frost are not really frequent (leaves damage, spike sterility only in some years)
•Moderate drought stress, occurrence depends by the year
•Low disease pressure.

Limitations of the Haymana station:

- No evaluation of late frosts damage
- No evaluation of germplasm response to abiotic stress combinations
- No possibility of performing accurate disease screening for mayor biotic constrains (rusts, scald, powdery mildew and net bloch), needs special arrangements and funds.

Winter barley

Locations	Activity	Stage of the material	Traits to be tested
Haymana, Konia, Eskiesehir (Turkey)	**Crossing, Selection,** Yield trials,	**Crossing block**, Segregating materials, preliminary and advanced Yield Trials,	Winter hardiness, early frost, grain yield
Maragheh, Iran	Evaluation,	Segregating materials	Winter hardiness, late frost, drought/heat
Kfardan, Lebanon	Yield trails International Nursery distribution	Observation Nurseries, Yield Trials, Observation Nurseries and seed multiplication	Yield potential, Biotic stresses

Köppen-Geiger climate classification (KGCC)

Haymana (Ankara, Turkey): classified as *Dsb* (snow, summer dry and warm dry)
with a cold temperate climate and with an average annual temperature of 10.1°C and average rainfall of 347mm.

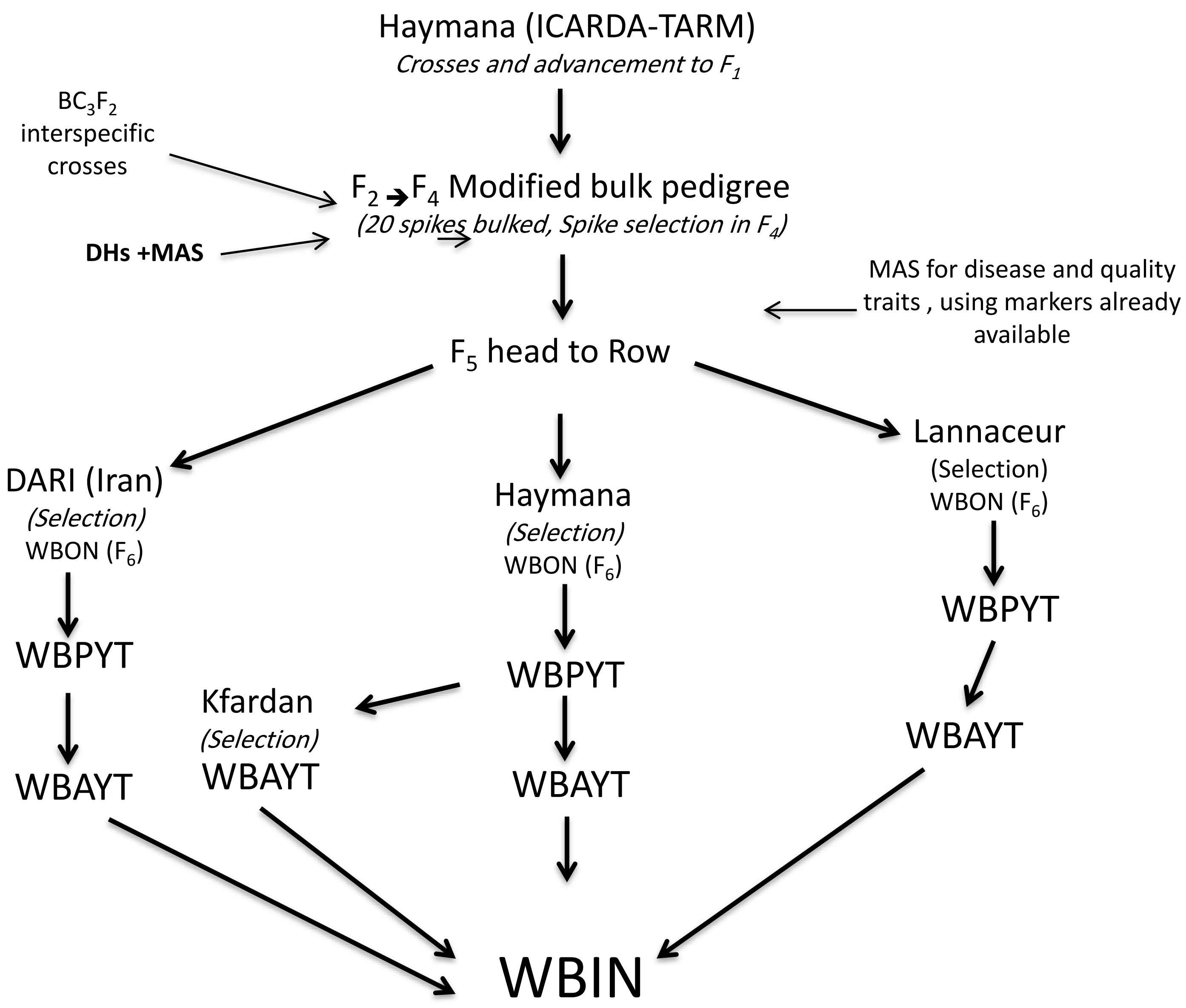
Maragheh (Iran): classified as local steppe climate and is classified as *BSk* (arid, summer dry and cold arid)
the average annual temperature is 11.2 °C and average rainfall of 353 mm.

Kfardan (Terbol, lebanon): classified as Csa (warm temperature, summer dry and cool summer) with an average rainfall of 300 mm, mainly concentrated during winter season, also characterized by cold/frost in winter season
and by terminal heat and drought stress. Average annual temperature 15 °C.

Winter barley

17Nursery	17Entry	CNAME	Hay (Kg/ha)	ESK (Kg/ha)	Kon (Kg/ha)
17WBAYT	301	**TARM92**	3335.00	4221.54	5140.0
17WBAYT	302	CWB117-77-9-7//Roho/Mazurka/3/Supie/4/Sararood	1741.67	3102.31	3571.7
17WBAYT	303	Kozir/Regina	3126.67	4415.38	5315.0
17WBAYT	304	Kozir/Regina	1765.00	3020.00	4824.2
17WBAYT	305	Sararood/3/YEA389-3/YEA475-4//Victoria/10/Excelle//CWB117-77-9-7/Sls/9/38P18/8/1/10	1721.67	3636.15	3571.7
17WBAYT	306	Pamir-147/Sonata/8/Alpha/Durra/7/P101/5/3896/1-15/3/3896/28//584/28/4/5050/6/Tipper	2533.33	2879.23	4760.0
17WBAYT	307	Pamir-147/Sonata/8/Alpha/Durra/7/P101/5/3896/1-15/3/3896/28//584/28/4/5050/6/Tipper	2158.33	3271.54	4681.7
17WBAYT	308	Pamir-147/Sonata/8/Alpha/Durra/7/P101/5/3896/1-15/3/3896/28//584/28/4/5050/6/Tipper	2358.33	4434.62	4819.2
17WBAYT	309	Hispanic/Sararood	2078.33	3850.77	3555.8
17WBAYT	310	Pamir-147/Sonata/8/Alpha/Durra/7/P101/5/3896/1-15/3/3896/28//584/28/4/5050/6/Tipper	2528.33	3151.54	3957.5
17WBAYT	311	Pamir-147/Sonata/8/Alpha/Durra/7/P101/5/3896/1-15/3/3896/28//584/28/4/5050/6/Tipper	2408.33	4602.31	3712.5
17WBAYT	312	Pamir-147/Sonata/8/Alpha/Durra/7/P101/5/3896/1-15/3/3896/28//584/28/4/5050/6/Tipper	1990.00	2786.15	4192.5
17WBAYT	313	**Larende**	3981.67	4406.92	5160.0
17WBAYT	314	Pamir-010/Sahara-3768/5/Roho//Alger/Ceres362-1-1/3/Tipper/4/Viringa'S'	2018.33	3766.92	2584.2
17WBAYT	315	CWB117-77-9-7//Hml-02/ArabiAbiad*2/5/WI2291//Apm/PI000046/3/Varunda/4/Alpha/3/Roho//Alger/Ceres/6/Tokak	2706.67	4035.72	3580.0
17WBAYT	316	CWB117-77-9-7//Roho/Mazurka/3/Supie/4/Sararood	1926.67	3580.77	3144.2
17WBAYT	317	Orsa/ICB100819//Sararood	2338.33	4103.85	3879.2
17WBAYT	318	Orsa/ICB100819//Gara Arpa	2453.33	4430.77	5190.8
17WBAYT	319	Aths/Lignee686/3/DeirAlla106/Lignee527//Asl/4/Lignee527/Rhn//Rhn-03/5/Mattina	2538.33	4369.23	5091.7
17WBAYT	320	Pamir-147/Sonata/8/Alpha/Durra/7/P101/5/3896/1-15/3/3896/28//584/28/4/5050/6/Tipper	2086.67	3787.69	4551.7
17WBAYT	321	Hispanic/Sararood	2513.33	3910.00	4010.8
17WBAYT	322	Zarjau/Regina	1955.00	3525.38	4445.8
		P101/5/3896/1-15/3/3896/28//584/28/4/5050/6/Tipper/7/Alpha/Durra/8/Roho//Alger/Ceres			

Winter barley: breeding strategy:



Haymana (ICARDA-TARM)

Crosses and advancement to F_1

BC_3F_2 interspecific crosses

DHs +MAS

$F_2 \rightarrow F_4$ Modified bulk pedigree

(20 spikes bulked, Spike selection in F_4)

MAS for disease and quality traits , using markers already available

F_5 head to Row

DARI (Iran)

(Selection)

WBON (F_6)

WBPYT

WBAYT

Haymana

(Selection)

WBON (F_6)

WBPYT

WBAYT

Kfardan

(Selection)

WBAYT

Lannaceur

(Selection)

WBON (F_6)

WBPYT

WBAYT

WBIN

Winter Barley International Nurseries

ICARDA International Nurseries Data Management System

Science for Better Livelihoods in Dry Areas

Seed counting, packaging and labeling machine

Winter Barley International Nurseries

Country	Institution	IBON	IBYT	Total
Afghanistan	[CARDA Coordinator Kabul	1	1	2
Algeria	Institut Technique des Grandes Cultures		2	2
Armenia	Ministry of Agriculture of RA	2		2
Bulgaria	Institute of Agriculture	1	1	2
China	Jiangsu Academy of Agricultural Sciences/ Biotechnology and Germplasm Resources Institute	1	1	2
China	Biotechnology and Germplasm Resources Institute	2	2	4
Greece	Geoponiko Spiti EPE	1	1	2
Hungary	Agricultural Research Institute of Hungarian Academy of Science	1	1	2
India	National Bureau of Plant Genetic Resources/ ICARDA-India	1	3	4
Iran	DARI/SPII	7	7	14
Iraq	Directortae of Agricultural Research	1	1	2
Jordan	NCARE	2	2	4
Morocco	INRA	1		1
Romania	National Agricultural Research and Development Institute Fundulea	1	1	2
Pakistan	Pakistan Agricultural Research Council	1	3	4
South Africa	ARC Small Grain Institute	1	1	2
Serbia	Biogranum Novi Sad	1	1	2
Tunisia	INRAT	2		2
Turkey	GAP	6	6	12
Uzbekistan	ICARDA-Tashkent for Uzbekistan, Tajikistan, Azerbaijan, Kyrgyzstan	7	7	14
		40	41	81

Genomic Selection: Introduction

Quantitative traits, are characterized by a large number of small effect loci, marker-assisted selection can harness only a limited portion of the genetic variation.

GS captures a much larger spread of genetic variation: because a large number of markers cover the entire genome, all chromosome segments are simultaneously considered for analysis to predict the breeding value of a given progeny line or genotype (Meuwissen et al. 2001).

GS captures all QTL effects irrespective of size by estimating allele effects of all markers simultaneously, using phenotypes and marker data of the training set (TS).

These effects are then used to estimate the breeding value of selection candidates, based solely on their allelic profile. The accuracy of GS is crucial for its success and effectiveness in a practical breeding scheme.

Genomic Selection: what do you need

Training population: phenotype + genotype

- How big? Approx. 100
- What to use? Half-sibs or Full-sibs

GEBV: genomic estimate of breeding value

- Replaces your phnotype 0-1
- i.e.: yield from 6 ton/ha to 0.78

Breeding population: genotype + select

- Opinions?

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Genomic Selection

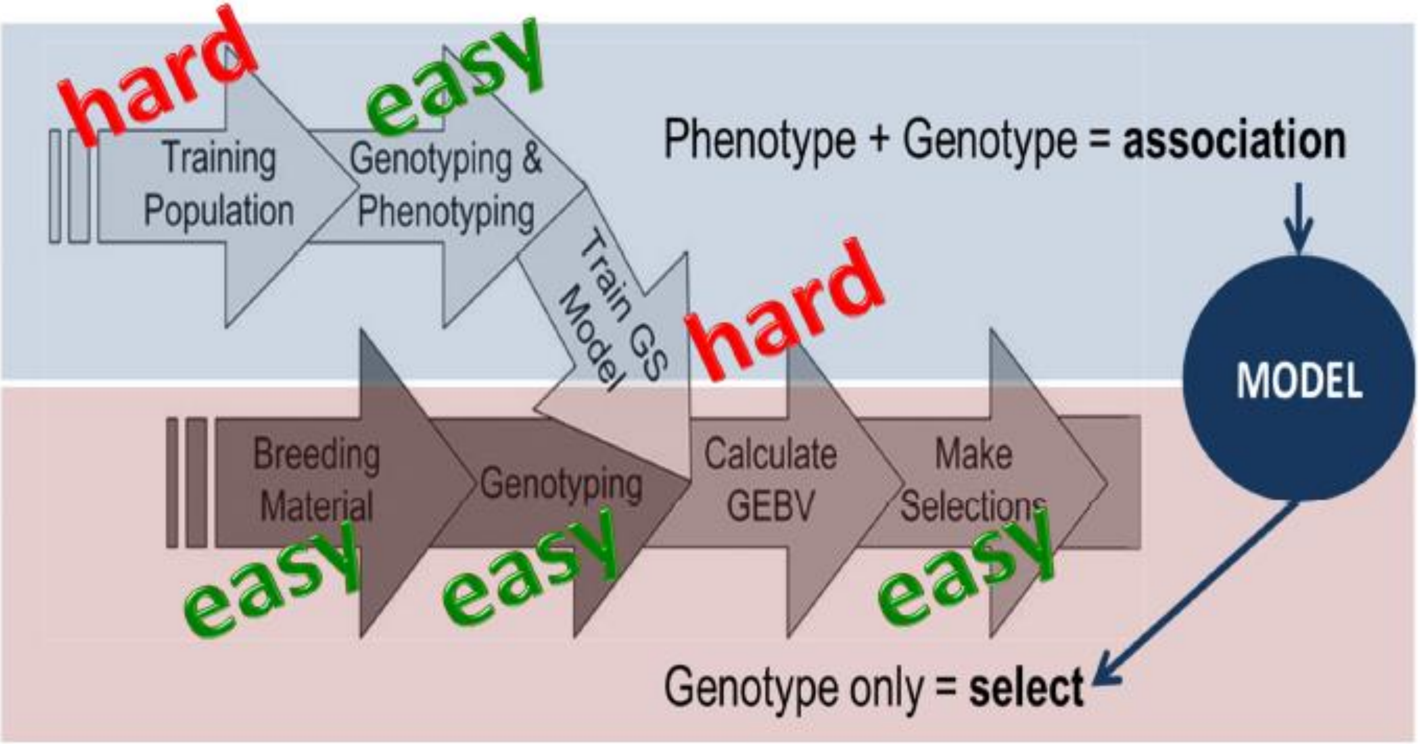
- Find markers – phenotype associations
- Use all markers for selection instead of the phenotype

To do it:

1- phenotype and genotype some (training population)

2- genotype all (breeding population)

3- Predict performance of “2” with “1”



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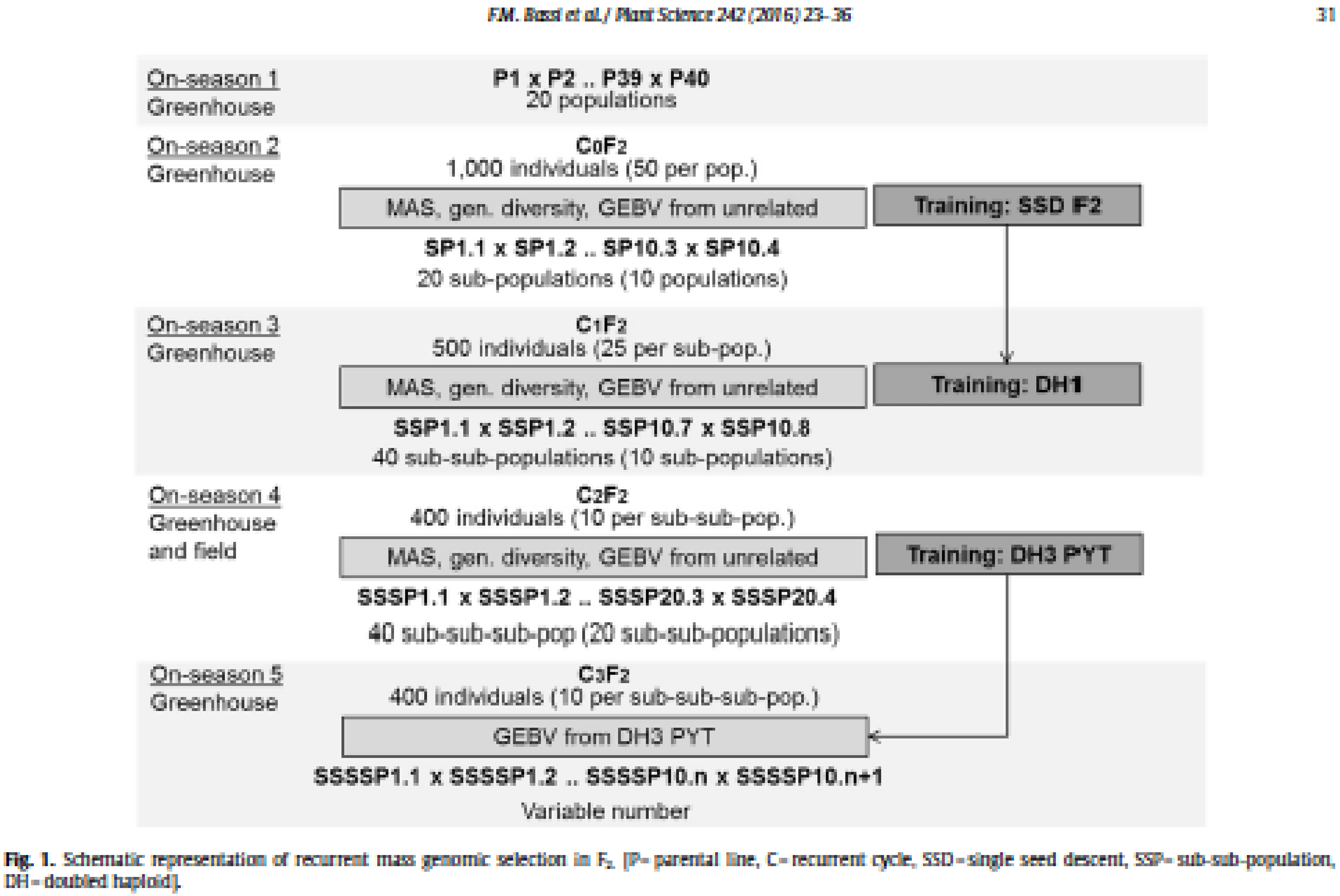
Genomic Selection



F.M. Bassi et al. / Plant Science 242 (2016) 23–36 31

On-season 1
Greenhouse

P1 x P2 .. P39 x P40
20 populations

On-season 2
Greenhouse

C_0F_2
1,000 individuals (50 per pop.)

MAS, gen. diversity, GEBV from unrelated

Training: SSD F2

SP1.1 x SP1.2 .. SP10.3 x SP10.4
20 sub-populations (10 populations)

On-season 3
Greenhouse

C_1F_2
500 individuals (25 per sub-pop.)

MAS, gen. diversity, GEBV from unrelated

Training: DH1

SSP1.1 x SSP1.2 .. SSP10.7 x SSP10.8
40 sub-sub-populations (10 sub-populations)

On-season 4
Greenhouse
and field

C_2F_2
400 individuals (10 per sub-sub-pop.)

MAS, gen. diversity, GEBV from unrelated

Training: DH3 PYT

SSSP1.1 x SSSP1.2 .. SSSP20.3 x SSSP20.4
40 sub-sub-sub-pop (20 sub-sub-populations)

On-season 5
Greenhouse

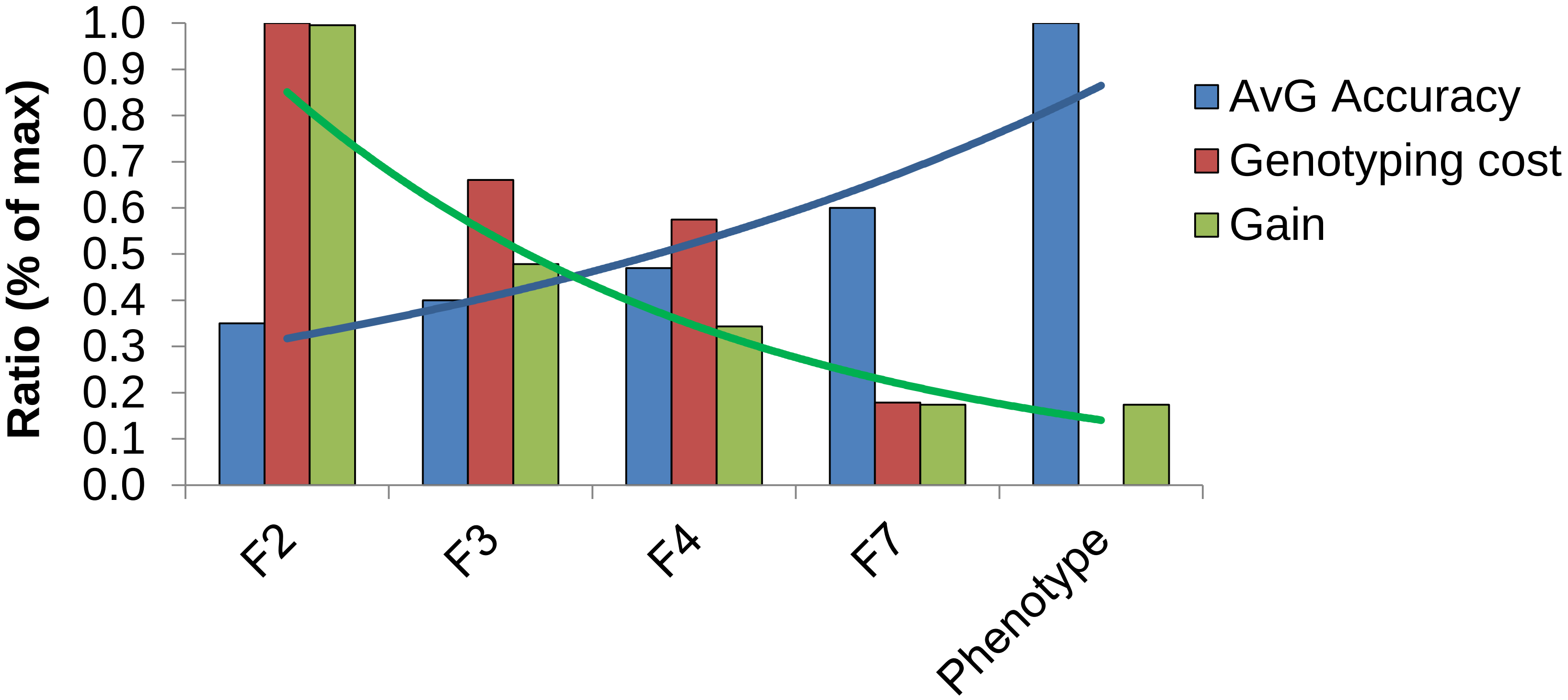
C_3F_2
400 individuals (10 per sub-sub-sub-pop.)

GEBV from DH3 PYT

SSSSP1.1 x SSSSP1.2 .. SSSSP10.n x SSSSP10.n+1
Variable number

Fig. 1. Schematic representation of recurrent mass genomic selection in F_2 (P = parental line, C = recurrent cycle, SSD = single seed descent, SSP = sub-sub-population, DH = doubled haploid).

Genomic Selection



- Recurrent schemes at different generations
- Assuming high accuracy in phenotypic selection
- Assuming higher accuracy when Full Sibs TP

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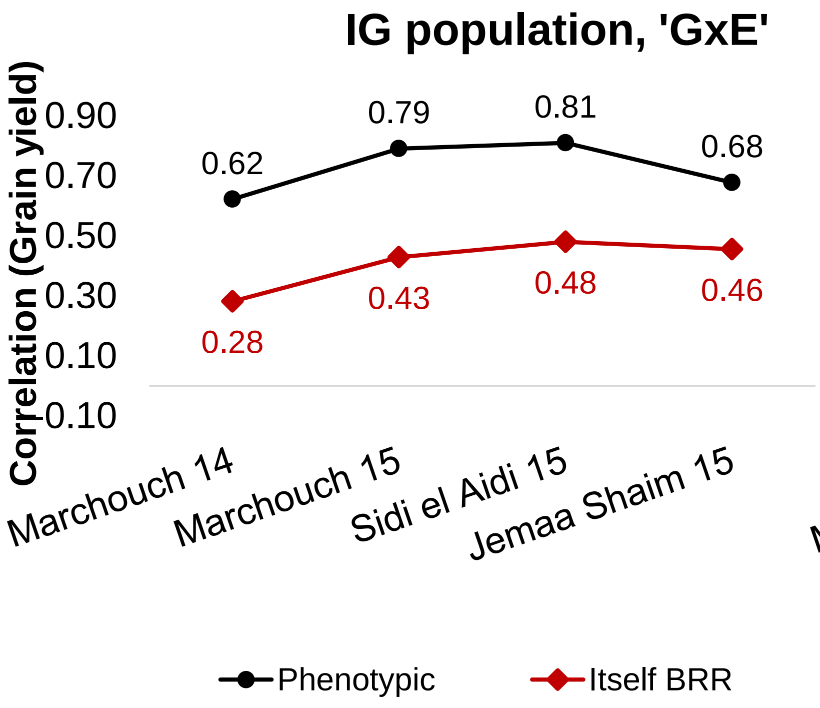
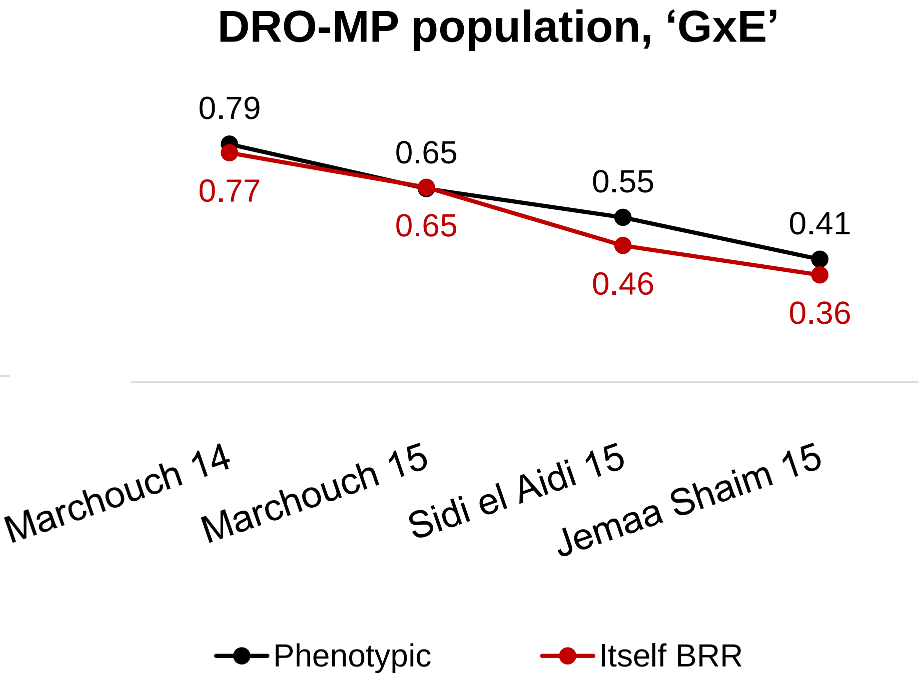
Genomic Selection

Training set predicting itself across environments using BLUE



SEVERITY OF DROUGHT STRESS



SEVERITY OF DROUGHT STRESS

BRR-BLUE model very good at predicting within the TP, but still worst than phenotype

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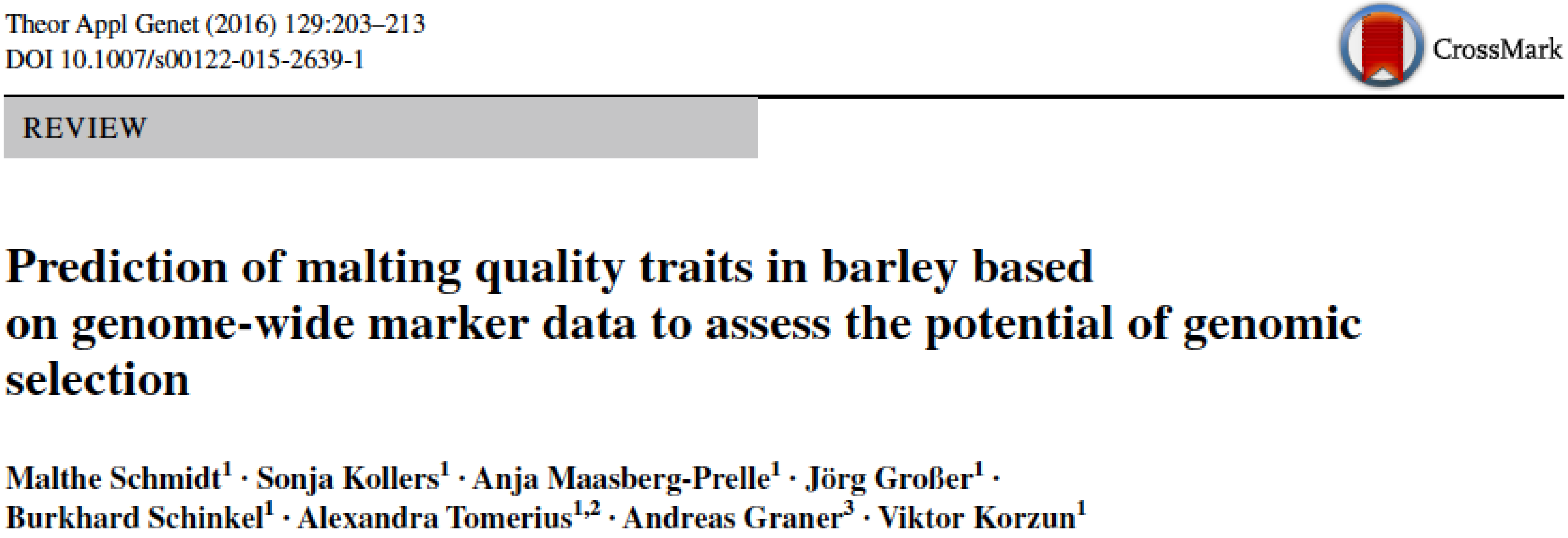
Genomic Selection: malting barley



Theor Appl Genet (2016) 129:203–213
DOI 10.1007/s00122-015-2639-1

CrossMark

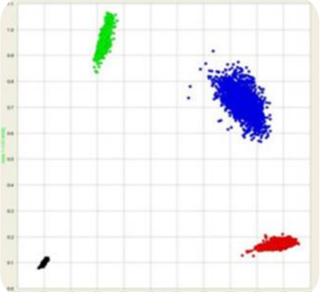
REVIEW

Prediction of malting quality traits in barley based on genome-wide marker data to assess the potential of genomic selection

Malthe Schmidt[1] · Sonja Kollers[1] · Anja Maasberg-Prelle[1] · Jörg Großer[1] ·
Burkhard Schinkel[1] · Alexandra Tomerius[1,2] · Andreas Graner[3] · Viktor Korzun[1]

The iBarMed Project: climate change and barley productivity

- Effects of climate change



"Looks like we'll bring your project in under budget. We were able to do it by not paying you."

WP1

Germplasm assembly for GS and association mapping

- Italy, Morocco, Spain, Tunisia,

WP2

Whole genome genotyping of genetic material

- Italy, Spain, Morocco, Tunisia

WP3

Multi environment field trials and trait phenotyping

- Italy, Morocco, Turkey, Tunisia

WP4

Data analysis and modelling for genomic prediction and association mapping

- Italy, Spain

WP5

Project management, dissemination of results and training of young scientists

- Italy, Spain, Morocco, Tunisia, Turkey

ICARDA Barley Breeding Team

Verma Ramesh (Ph.D., Senior Barley Breeder, program coordinator)
Andrea Visioni (Ph.D.,Associate winter barley)
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Thanks for your attention!