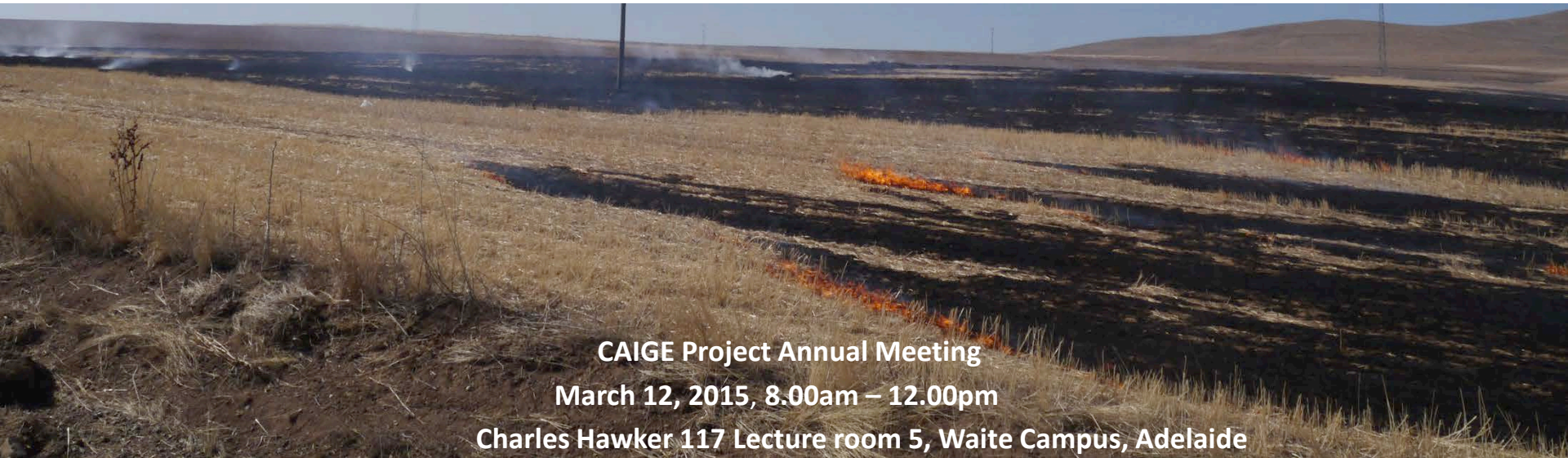
GRDC Grains Research & Development Corporation

CIMMYT

Identification and utilization of novel sources of resistance to crown rot and the root lesion nematodes in adapted spring and durum wheat

Amer Dababat



CAIGE Project Annual Meeting

March 12, 2015, 8.00am – 12.00pm

Charles Hawker 117 Lecture room 5, Waite Campus, Adelaide

2013-2014 Work Plan

1. A total of 265 SW were screened for RLN and Cr
2. 208 DW from CIMMYT –Mexico were screened against RLN and Cr
3. 3094 lines from the Turkish landraces were screened for Cr under the greenhouse conditions.
4. Two subsets of the Turkish Landraces were selected based on FIGS by Ken Street one against Cr and the 2nd against RLN and were screened under growth room conditions
5. 30 lines from CRI project were screened against Cr under different environmental conditions
6. 82 Australian varieties were screened

CR SCREENING METHODOLOGIES

A) For seedling screening grown under Growth room conditions:

1) 1 ml ($1x10^6$ spore/ml) spore suspension inoculated to the 1 week old seedling stem base.
2) 8 weeks later plants are harvested and score is given depending on the browning percentage on the crown, according to 1-5 scale. 1: % 1-9 (R), 2 : %10-29 (MR) 3: % 30-69 (MS) 4: % 70-89 (S) 5: % 90-99 (HS)
3) 5 to 7 replicates are used for each line. Each trial is repeated once
4) Plant growing under growth room conditions of 23 C ± 2, 16 h artificial light, 70 to 80 % relative Humidity, and both up and below watering are applied.

B) For Adult plant resistance under field condition:

Seeds are sown in 1 m long with 3 reps.
Inoculum - in addition to the natural infection in the field each row (1 m long) receives 1 tea spoon of wheat bran is added as additional inoculum at sowing time (seed and then both seed and inoculum are covered spontaneously with soil).
The following are measured:
1) **Growth Reduction - usually at 3-4 tiller stage**
1 = < 5-10 % , 2 = > 5-10 %, 3 = 10-30 %, 4 = 30-50 %, 5 > 50 %
2) **White Head formation post anthesis**
White Head is followed very carefully after heading stage to capture the whitening on the spikes. This year we added a new facility in order to better observe the WH symptoms by planting under the greenhouse (as an adult screen, similar to the SARDI).
1 = < 5-10 % , 2 = > 5-10 %, 3 = 10-30 %, 4 = 30-50 %, 5 > 50 %
3) **Crown Scoring post anthesis**
At harvest time, 30 tillers (10 for each replicate) are sampled and Crown scores are given for each tiller based on browning on the stem base
1-5 scale. 1: % 1-9 (R), 2 : %10-29 (MR) 3: % 30-69 (MS) 4: % 70-89 (S) 5: % 90-99 (HS)
4) **Yield Trial**
To evaluate their yield potential performance - for the best Spring Wheat lines are planted in plots (4 rows 2 m long). X 3 reps X +/-. A number of phenotypic scores are also taken.

Root Lesion Nematodes (P. neglectus and P thornei)

1. Surface sterilized seeds are germinated onto filter paper placed in Petri dishes and kept moistened with sterile water
2. Best 7 germinated seeds are planted
3. Pre-germinated seeds of about 3 cm root in length are planted in tubes 3x13 cm consisting of soil combination (70:29:1 Sand: Field soil: organic matter)
4. 400 nematodes for each tube are inoculated in three holes around the stem 7 days after sowing. RLN culture is maintained on carrot media
5. 9 weeks after nematode inoculation plants are harvested and nematode are extracted from both roots and soils and counted
6. CHECK LINES: Australian check lines from the ring test are used to compare the new screened lines a few local checks are used as well
7. Rank is given from 1 to 5 and according to the resistant & susceptible check lines used as follow 1=R, 2=MR, 3=MS, 4=S, and 5= HS

Summary of 2014 Spring Wheat Screening Nursery

RLN-Pn = 14 MR lines
RLN-Pt = 11 MR lines
} 8 lines in common

Cr = 31 MR lines
} 9 in common with RLN

They are planted in Yield Trials in 2015

Summary of 2014 Durum Wheat Screening Nursery

RLN-Pn = 11 MR lines
RLN-Pt = 12 MR lines

Cr = 36 MR lines

They are planted in Yield Trials in 2015

CAIGE Project

CIMMYT Australia ICARDA Germplasm Evaluation

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GO

Latest News

- Crown Rot resistant recommended lines
- 2015 Imported material
- Annual CAIGE meeting 2015
- Data from Trials 2014
- Rust data from PBI

SBP resistant lines - 2015 imports

Spring Wheat selections from CIMMYT-Turkey

SPRING WHEAT SELECTIONS by Amer Dababat - SWM15

Durum wheat selection from CIMMYT - Turkey

Selections by Amer Dababat - DCR15

SBP data

- SBP project intro
- SPB 2009 Nursery
- SPB 2010 Nursery
- SPB 2011 Nursery
- SBP - 2012 selections
- SBP - 2013 selections
- SBP - 2014 selections
- SBP - 2015 imports

Imported Material

- Wheat
- Barley
- Durum
- Chickpeas

Evaluation

- Donor Data
- CAIGE Yield Trials
- Disease Screening
- Genotyping

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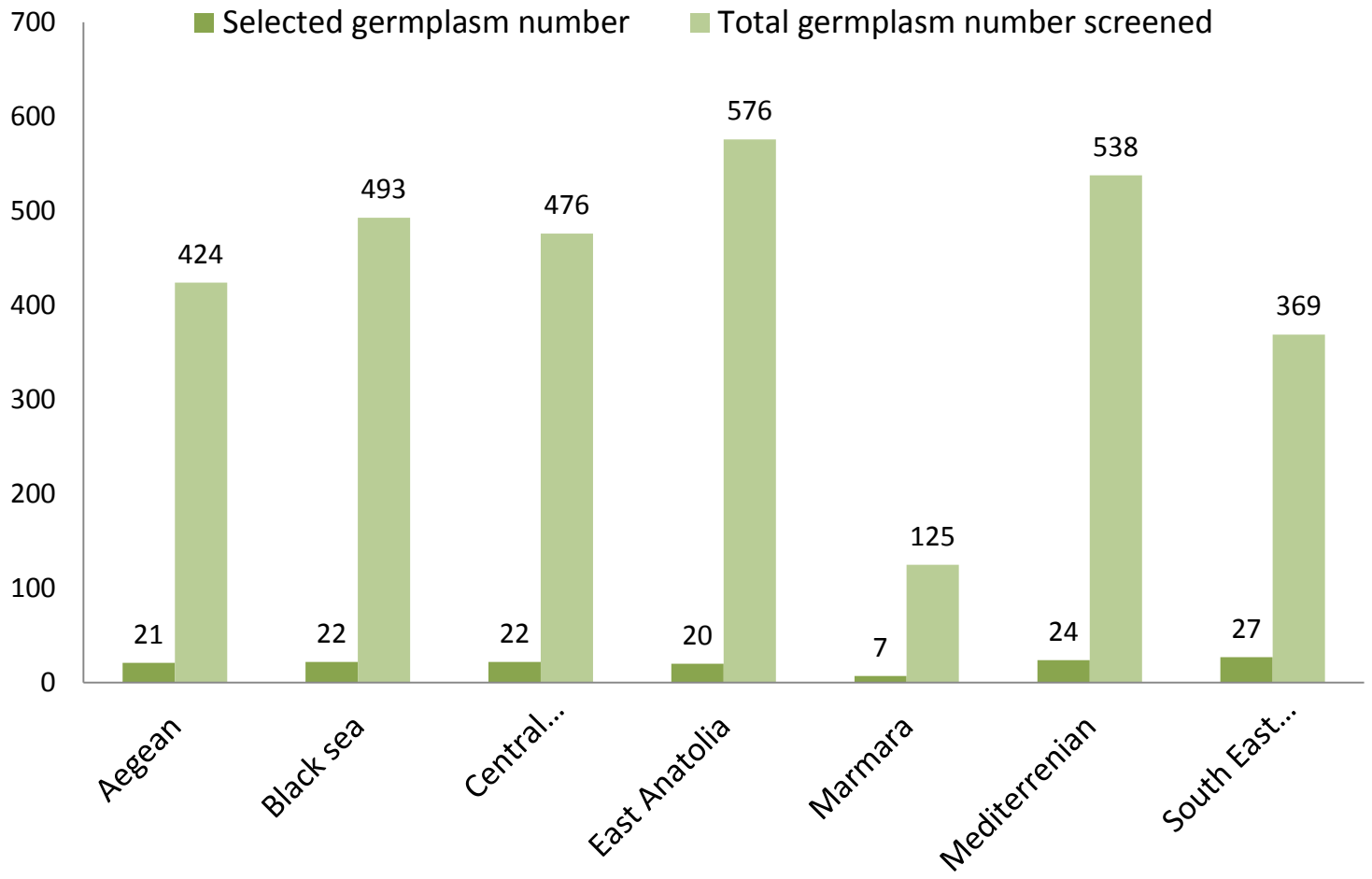
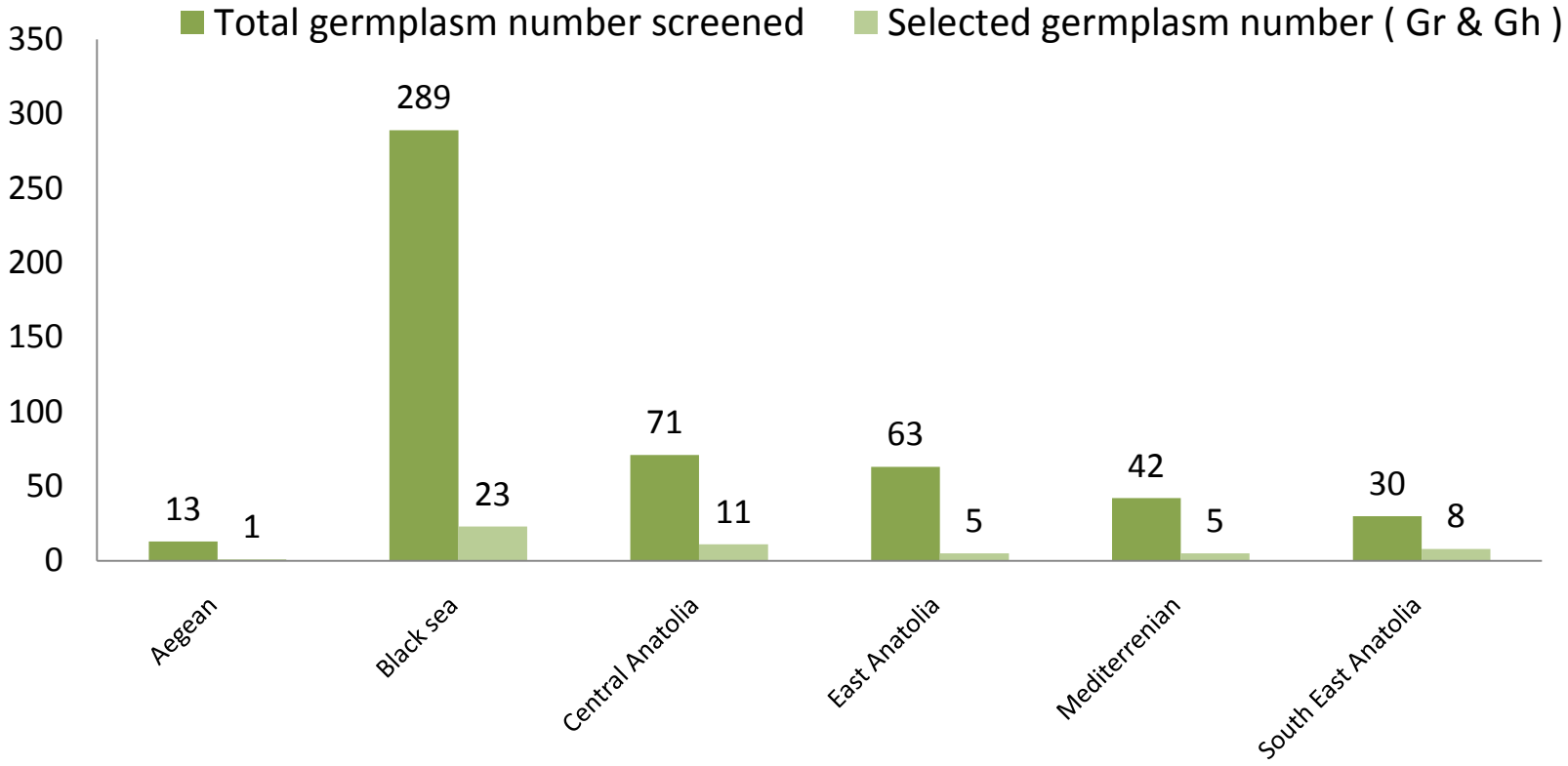
Turkish Landraces Reaction for CR at Adult Stage



Totally 3094 germplasm tested under Gh conditions against CR (3001 landraces + 93 National material)

The number of germplasm selected for CR from Turkish LR-FIGS



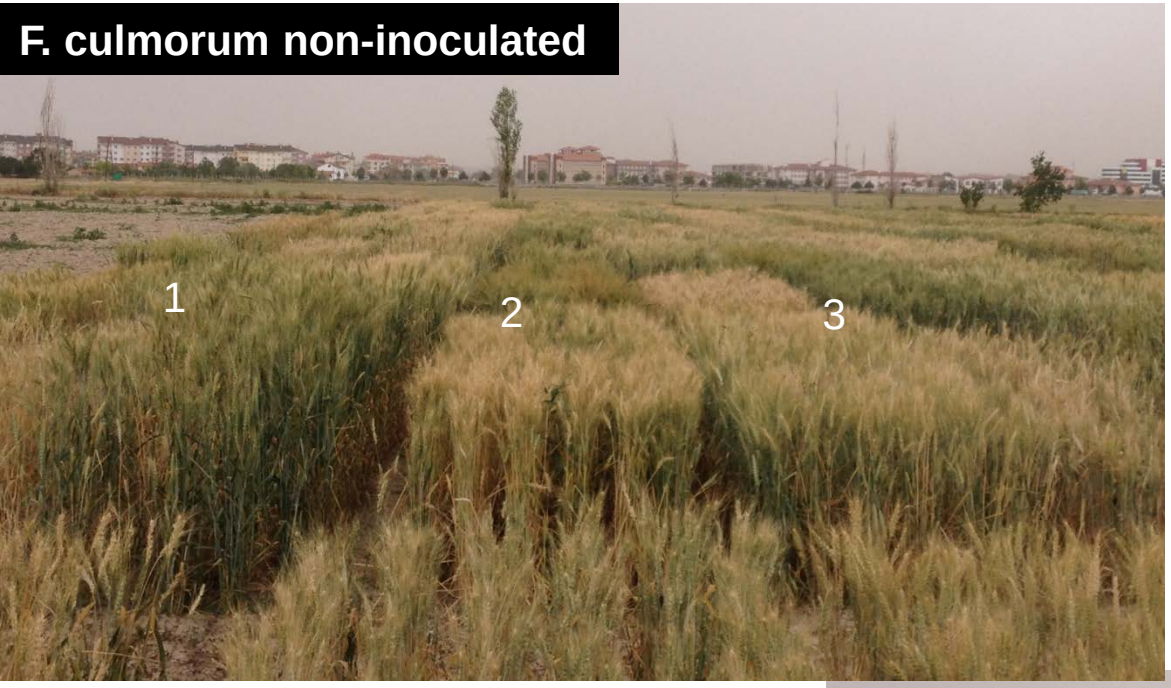
Totally 508 germplasm tested under GR & GH conditions against Cr

Crown Rot Initiative Project – Sydney Uni

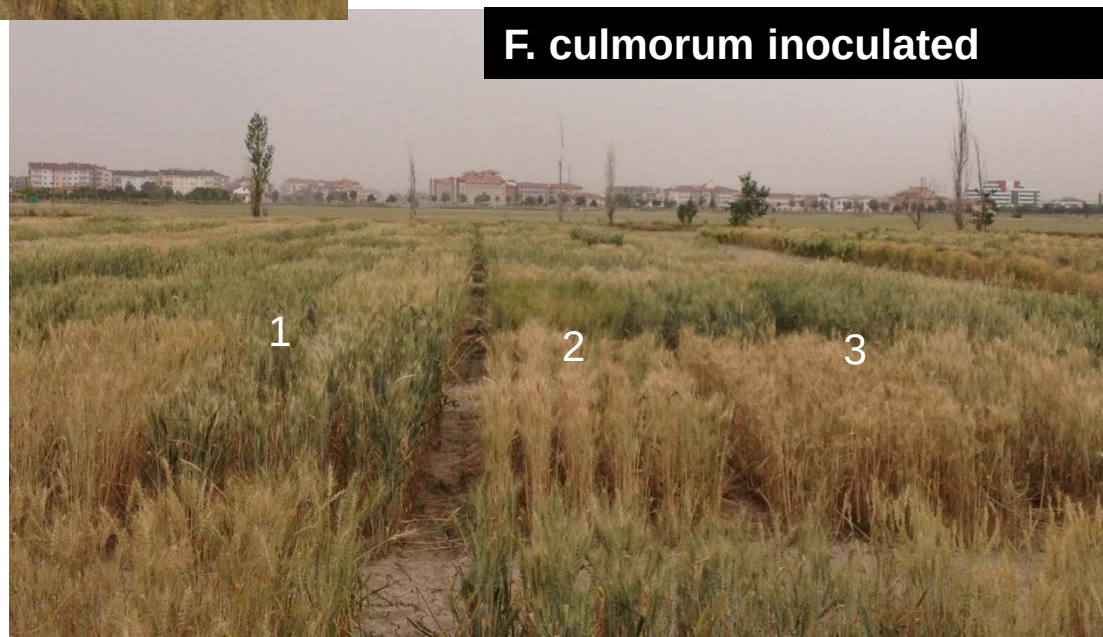
			2014 GR-CR (1st Run)	2014 GR-CR (2nd Run)	2014 GH-CR	2014 F-CR-Fc (Yoz)	2014 F-CR-(Kon)	2014 F-Plant Height (Ed)
Selection (Gr and/or Gh)	Ent 14	C-Name	Mean	Mean	Mean	Mean	Mean	PH (cm)
cr	1	SUNCO/SYN110	2	2.3	3.0	2.3	2.3	102
cr	4	SUNCO/SYN110	2.4	3.0	2.0	2.3	2.3	102
cr	6	SUNCO/SYN110	2.6	2.0	2.3	2.3	2.3	93
cr	7	SUNCO/SYN110	3.2	2.4	2.0	1.7	1.7	102
cr	9	JANZ/SYN110	1.4	1.0	2.0	2.0	1.7	122
cr	12	JANZ/SYN110	1.2	1.2	3.3	2.3	3.0	96
cr	13	JANZ/SYN110	1.8	1.6	2.0	2.7	2.7	112
cr	19	EGA GREGORY///CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR	1	1.4	2.3	2.7	2.7	113
cr	20	EGA GREGORY///CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR	2	2.2	2.0	3.0	3.0	107
cr	21	EGA GREGORY///CROC_1/AE.SQUARROSA (224)//OPATA/3/PASTOR	2	1.2	1.7	3.0	3.3	119
		EGA GREGORY///CROC_1/AE.SQUA				3.0		

CR Yield Trials in 2014 - KONYA

F. culmorum non-inoculated



F. culmorum inoculated



SBP CIMMYT-Turkey, 2014

The selected germplasm from 14AUSTRALIA (#31..112) nursery under growthroom and greenhouse conditions.

14AUS Ent #	Name	Seedling Stage	Adult Stage
47	EGA Bonnie Rock	MR/MS	MR/MS
62	GBA Sapphire	MR	MR/MS
77	Mackellar	MR	MR
81	Naparoo	MR	MR/MS
83	Phantom	MR	MR/MS
88	Spitfire	MR	MR/MS
89	SQP Revenue	MR	MRMS
94	Sunvale	MR/MS	MR
95	Sunvex	MR/MS	MR
103	EGA Wylie	MR	MR

A total of 82 entries of AUS germplasm are planted in Turkey in 2015.

Incoming seed expenses

In-coming Seed expenses (TL)	Average	Min	Max
pre-import permission	400	400	400
Inspector	165	110	220
custom agency	300	300	300
Storage in custom	250	100	400
Tax	77	77	77
Tansfer from Istanbul to Ank	200	100	300
Shipment Cost	600	400	800
Shipment to the Institute	100	50	150
2nd Inspector	150	100	200
TOTAL (TL)	**2242**	**1637**	**2847**
TOAL (AUS $)	**1124**	**821**	**1429**

Note: personnel and office expenses NOT included

The following people who received the seed:

Name	ZAD13 lines	SBP12 lines
#Lines	**40 Lines**	**192 lines**
DAVIES, Phil	30	108
HERDE, Damien	23	88
JACOBS, Bertus	32	114
LU, Meiqin	27	108
MARES, Daryl	35	158
Shankar, Manisha	35	158
SNYMAN, Lisle	26	100
TAN, Kar-Chun	28	108
TOKACHICHU, Raju	34	114

Lines Selected for crossing in 2014 by SBP (CAIGE website)

2014 - #		Cross	CID	SID	GID	ON	Pn	Pt	Cr	Ha
1	3	KLDR/PEWIT1//MILAN/DUCULA	462712	61	5686762	SEED2010YM	x	x	x	
2	5	MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	476179	65	5894993	SEED2010YM	x	x	x	
3	8	D67.2/P66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/PASTOR/SLVS	481431	274	5895245	SEED2010YM	x	x	x	
4	18	PASTOR/JANZ//BERKUT	462770	19	5686795	SEED2010YM	x	x	x	
5	23	SOKOLL/EXCALIBUR	479309	358	5687026	SEED2010YM	x	x	x	
6	26	SOKOLL//SW89-5124*2/FASAN	485799	45	5894621	SEED2010YM	x	x	x	
7	37	SOKOLL//W15.92/WBLL1	473237	30	5435851	IRANGholamAhmadi 2010	x	x	x	
8	5	NSM*4/14-2//FRTL/2*PIFED/3/VORB	512786	100	6000219	29th SAWSN	x	x	x	
9	13	ACHTAR/4/MILAN/KAUZ//PRINIA/3/BAV92	504457	64	6000537	29th SAWSN	x	x	x	
10	28	SOKOLL//FRTL/2*PIFED	495447	74	6001172	29th SAWSN	x	x	x	
11	60	EGA BONNIE ROCK*2/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	512870	88	6000085	29th SAWSN	x	x	x	x
12	63	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/5/MONARCA F2007/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1	512926	130	6000104	29th SAWSN	x	x	x	x
13	81	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	506887	99	6000615	29th SAWSN	x	x	x	x
14	98	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	506830	97	6001012	29th SAWSN	x	x	x	
15	111	D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/WBLL1*2/TUKURU	495387	101	6001138	29th SAWSN		x	x	x
16	112	BAV92/SERI	285851	1380	6001233	29th SAWSN	x	x	x	
17	123	SOKOLL*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/STAR	496027	102	6001642	29th SAWSN	x	x	x	x

Root Lesion Nematodes (Pt/Pn) were screened in growth room
Crown rot (Cr) were screened in growth room, greenhouse, field conditions

SW crosses 2014

Ent	Cid	Sid	GID	F_Cross_Name	F_GID	F_Ent	M_Cross_Name
6101	637874	0	7454630	EGA BONNIE ROCK*2/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	6000085	514	MACE
6102	637875	0	7454631	EGA BONNIE ROCK*2/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	6000085	514	SCOUT
6103	637876	0	7454632	EGA BONNIE ROCK*2/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	6000085	514	SUNTOP
6104	637877	0	7454633	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/5/MONARCA F2007/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1	6000104	515	MACE
6105	637878	0	7454634	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/5/MONARCA F2007/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1	6000104	515	SCOUT
6106	637879	0	7454635	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/5/MONARCA F2007/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1	6000104	515	SUNTOP
6107	637880	0	7454636	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	6000615	516	MACE
6108	637881	0	7454637	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	6000615	516	SCOUT
6109	637882	0	7454638	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	6000615	516	SUNTOP
6110	637883	0	7454639	SOKOLL*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/STAR	6001642	520	MACE
6111	637884	0	7454640	SOKOLL*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/STAR	6001642	520	SCOUT
6112	637885	0	7454641	SOKOLL*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/STAR	6001642	520	SUNTOP
6113	637886	0	7454642	KLDR/PEWIT1//MILAN/DUCULA	5686762	504	MACE
6114	637887	0	7454643	MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	5894993	505	SCOUT
6115	637888	0	7454644	PASTOR/JANZ//BERKUT	5686795	507	MACE
6116	637889	0	7454645	SOKOLL/EXCALIBUR	5687026	508	SCOUT
6117	637890	0	7454646	SOKOLL//SW89-5124*2/FASAN	5894621	509	SUNTOP
6118	637891	0	7454647	SOKOLL//W15.92/WBLL1	5435851	510	MACE
6119	637892	0	7454648	NIGHTJAR	6000219	511	SCOUT
6120	637893	0	7454649	ACHTAR/4/MILAN/KAUZ//PRINIA/3/BAV92	6000537	512	SUNTOP
6121	637894	0	7454650	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	6001012	517	SCOUT
				D67.2/PARANA 66.270//AE.SQUARROSA			

DW crosses 2014

A total of 325 crosses made:
Australian Parents (B04-17, CAPAROI, HYPERNO, JANDAROI, TJILKURI, WID802, and YAWA)

1. **Australian Elite Cultivars x Best SBP RESISTANCE (111x)**
2. **Intercross of Best Crown Rot 2014 (36x)**
3. **CIMMYT Elite Lines x Best SBP Resistance (149x)**
4. **Best All SBP 2014 X Best Crown Rot 2013 (29x)**

December 2017 , F6 will be in HR in Turkey

Turkish Landraces x Australian DW and SW crosses

Nominated Australian lines:

DW: CAPAROI, JANDAROI, TJILKURI, WID802

SW: SUNTOP, SPITFIRE, MACE, WALLUP, SCOUT, EMU ROCK

Turkish Landraces: best lines resistant to Cr and /or RLN based on screening an original nursery of 3094 lines and the subset set recommended by Ken Street based on FIGS

New DH population

SOKOLL*2/4/CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/STAR X **SUNTOP**

F1 seeds will be sent in 2015 to Mexico, a min of 200 seeds will be sent.

2015 Work Plan

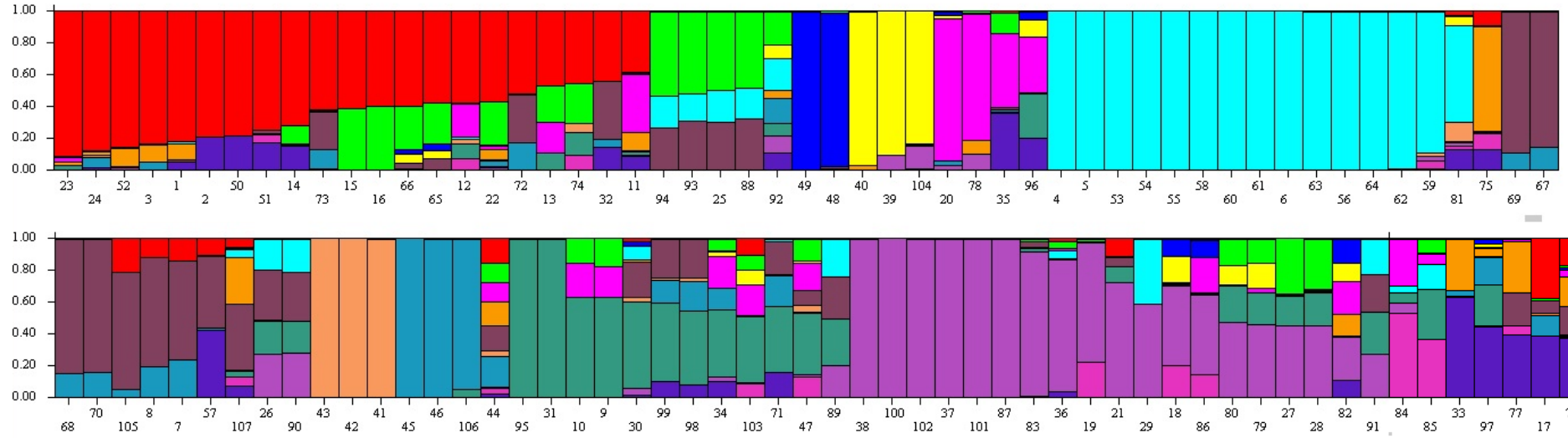
#	Nursery	Ent #	GH[a]	Growth room		Field SBP	
			CR	RLN	CR	RLN	CR
1. GRDC							
19	2015 SW	169	x	x	x		x
20	2015 DW	200	x	x	x		x
21	YT SW14 CR	40					x
22	YT DW14 CR	48					x
23	YT SW14 RLN	22				X	
24	YT DW14 RLN	20				X	
25	14 SBP-YT CR	40					X
26	14-29th SAWSN_RLN	32				X	
27	Crosses SW MexxSWAus	Parents from Australia (6) and CIMMYT(6) used to make at least 50 crosses with CIMMYT SBP nominated germplasm					
28	Crosses DW Mex x DW Aus	Parents from Australia (5) nominated to make targeted crosses with CIMMYT SBP nominated germplasm					
29	Landraces	3094					
2. SBP – Sydney (CRI)							
31	CRI -YT	13					x
3. SBP (Miscellaneous)							
34	Mark Dieters – CR10	72	x		x		
36	Joe's Exp PhD	20					

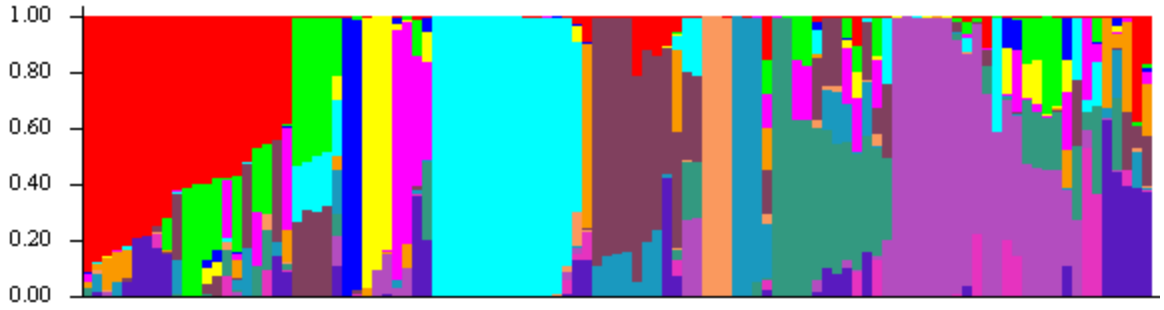
Sick Plot Establishment / Eskisehir

1. CCN – Hf
2. RLN – Pt
3. CR - Fc

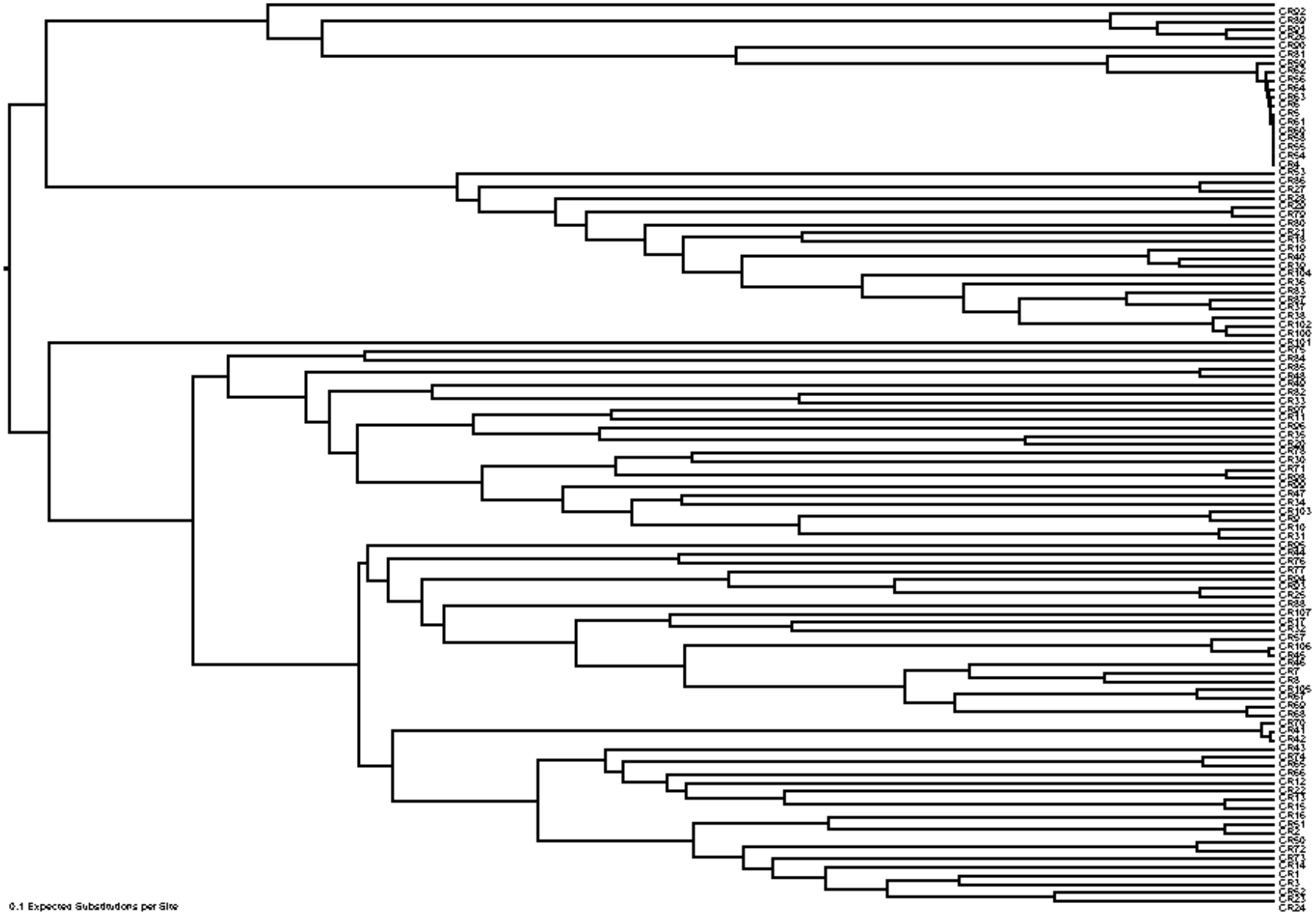
Association Mapping Study

Population Structure of the 107 CR Resistant lines





Phylogenetic tree of the 107 CR resistant lines (UPGMA)



Markers Associated with CR

Trait	marker	chr.	positon	F	p	minus LOG	markerR2	Genetic Var	Residual Var
CR3	wPt-731132	7D	170.7	11.78774967	8.99E-04	3.046116139	0.10049984	0.17061751	0.180096664
CR3	wPt-3435	4A	38.4	11.24456453	0.00116521	2.933595878	0.09586876	0.17061751	0.180096664
CR1	wPt-8000	4A	81.9	10.10312365	0.002022961	2.694012477	0.09803126	0.07509734	0.126097973
CR2	tPt-7068	3B	79.3	9.903512017	0.002230207	2.651654789	0.10382194	0.14157836	0.143168688
CR1	wPt-667155	1A	13.5	9.749281161	0.002405311	2.61882883	0.0945979	0.07509734	0.126097973
CR4	wPt-5029	2A	46	9.687099176	0.002479878	2.60556972	0.0873981	0.07640831	0.319255954
CR3	wPt-7251	7B	224	9.598127812	0.002590756	2.58657355	0.08183159	0.17061751	0.180096664
CR3	wPt-733764	6A	24.2	9.579196516	0.002615003	2.582527772	0.08167019	0.17061751	0.180096664
CR3	wPt-5283	7B	84.6	9.54119705	0.002664385	2.574402943	0.08134621	0.17061751	0.180096664
CR3	wPt-8490	2A	28.9	9.393027611	0.002866351	2.542670561	0.08008295	0.17061751	0.180096664
CR2	wPt-744300	7D	4.5	9.189920856	0.003169295	2.499037293	0.09634112	0.14157836	0.143168688

Association analysis of the resistance to cereal cyst nematodes '*Heterodera avenae*' and root lesion nematodes '*Pratylenchus neglectus* and *P. thornei*' in semi-arid CIMMYT's advanced spring wheat lines

Summary:

- 11 markers to *H. avenae*.
- 25 markers to *P. neglectus*
- 9 markers to *P. thornei*.

Novel QTL's were found on chromosomes:

- 5A, 6A, and 7A for *H. avenae*
- 1A, 1B, 3A, 3B, 6B, 7AS and 7D for *P. neglectus*
- 1D, 2A, and 5B for *P. thornei*

Capacity Building

Shree Ram Pariyar	PhD	Analysis of Wheat Resistance to *Heterodera filipjevi* through association mapping	Bonn Uni Germany	2011-2015
Fateh Toumi	PhD	Control of Cereal Cyst Nematode in Syria through Identification and Characterization of Genetic Resistance for Wheat Improvement	Ghent Uni Belgium	2011-2015
Samad Ashrafi	PhD	Using Naturally Occurring *Endophytic Fungi* and/or *Bacteria* Associated with Wheat as a Biological Control Agent against the Cereal Cyst Nematodes, *Heterodera* species	Kassel Uni Germany	2013-2016
Elfinesh S. Gebremariam	PhD	Occurrence, distribution and frequency of the dry land Crown rot caused by *Fusarium culmorum* in three main wheat grown regions in Turkey	Ankara Uni and WSU	2013-2015
Amangeldi Nurgul	PhD	Occurrence and distribution of the dryland crown rot caused by Fusarium species in spring wheat growing areas in Central and Northern Kazakhstan	KazNAU	2015 - 2018
Abdukerim Rauza Zhusipkyzy	PhD	The cereal cyst nematodes caused by Heterodera species in spring wheat growing areas in Central and Northern Kazakhstan	KazNAU	2015 - 2018
Mahin Ahmadi	PhD	The interaction of biotic and abiotic stresses on wheat	Shahrekord Uni	2015 - 2018
Lea Meagher	PhD	Mechanisms of action of different wheat resistance QTL to the root-lesion nematodes Pratylenchus thornei and Pratylenchus neglectus in lines from Australia and the CIMMYT program in Turkey	Uni of Southern QLD	2015 - 2018
Joseph Barry	PhD	A comparison of the growth patterns of three root pathogens in wheat	Uni of Southern QLD	2014 - 2018

PUBLICATIONS

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