

Chickpea and Flax Breeding and Genetics at CDC: a journey of two superfood

Bunyamin Tar'an

Acknowledgments:

Sub program chickpea breeding:

(share responsibility with pea, lentil, bean & faba bean programs)

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CDC Pulse Pathology Program
CDC Cereal and Flax Pathology Program
All collaborators at CDC and U of S
All former students, technicians and postdocs
National and International collaborators

Superfood claims!

Popular Latest Newsletters *The Atlantic* Sign In

HEALTH

In the Future, Everything Will Be Made of Chickpeas

America is finally embracing an ingredient that much of the world has relied on for millennia.

By Amanda Mull



get / Getty

MARCH 14, 2019

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SPEAKING OF HEALTH

Superfood of the Month: Chickpeas

Chickpeas are incredibly easy to include in your diet because they are affordable and available in most grocery stores.



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Superfood spotlight: cooking with chickpeas

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SPEAKING OF HEALTH TUESDAY, MARCH 31, 2015

Flaxseed is nutritionally powerful



TOPICS IN THIS POST

[Nutrition](#) [Get Healthy Recipes And Tips](#)

Flaxseed may be small, but its health benefits are big. It contains numerous salubrious components, with highlighted nutrients being [omega-3 fatty acid](#) ALA (alpha-linolenic acid), fiber and lignans.

Omega-3 fatty acids are good fats that may help lower total cholesterol and low-density lipoprotein (LDL or bad) cholesterol levels, reduce inflammation and reduce the risk of certain cancers. Fiber can help relieve constipation, control cholesterol levels and keep you feeling full longer. Flaxseed also contains lignans, which provide antioxidant protection.



Superfood claims!

INGREDIENT INTELLIGENCE

The Original Superfood: Flax Seeds



By [Emma Christensen](#)
Updated May 2, 2019



(Image credit: [Apartment Therapy](#))

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Superfoods

Linseeds

Golden linseeds (also known as flaxseeds) are powerhouses of nutrition. They have a subtle nutty, slightly earthy flavour and are a cheap superfood that everyone can include in their diet. They are available whole or - for easier absorbtion - split, ground or as linseed or flaxseed oil.

Protein and Oil contents of chickpea germplasm collection and elite lines/released cultivars across 3 locations in SK (2017-2019)

Trait	Population	N	Range	Mean	SD
Protein (% DWB)	Germplasm	184	13.6 - 27.5	19.5	2.5
	Varieties	100	13.5 - 27.3	19.0	2.1
Oil (% DWB)	Germplasm	184	2.5 - 9.4	6.4	1.0
	Varieties	100	3.5-10.2	6.7	0.8

Source: Orsak, 2022

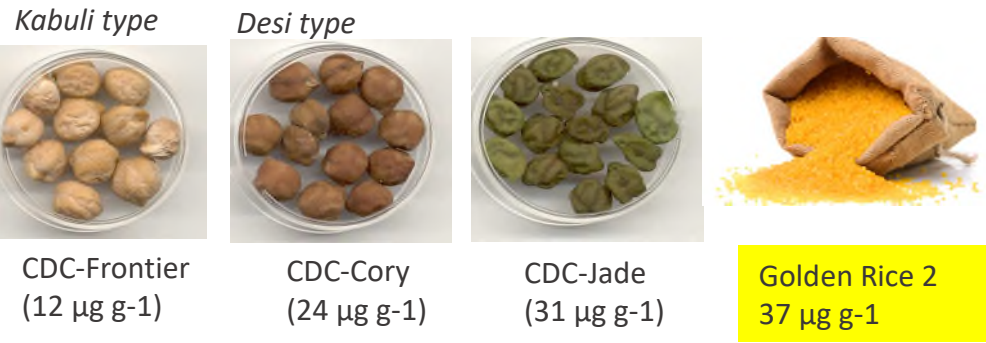
Mineral Micronutrient Content of Cultivars of Field Pea, Chickpea, Common Bean, and Lentil Grown in Saskatchewan, Canada

Heather Ray, Kirstin Bett, Bunyamin Tar'an, Albert Vandenberg, Dil Thavarajah, and Thomas Warkentin*

Table 8. Chickpea (*Cicer arietinum*) cultivars and mineral contents. Cultivars found not to be significantly different by Tukey's test (0.05 level) are indicated by same letter, within each column. For Mg, Fe, Zn, Mn, Cu, and Se there were no pairwise significant differences.

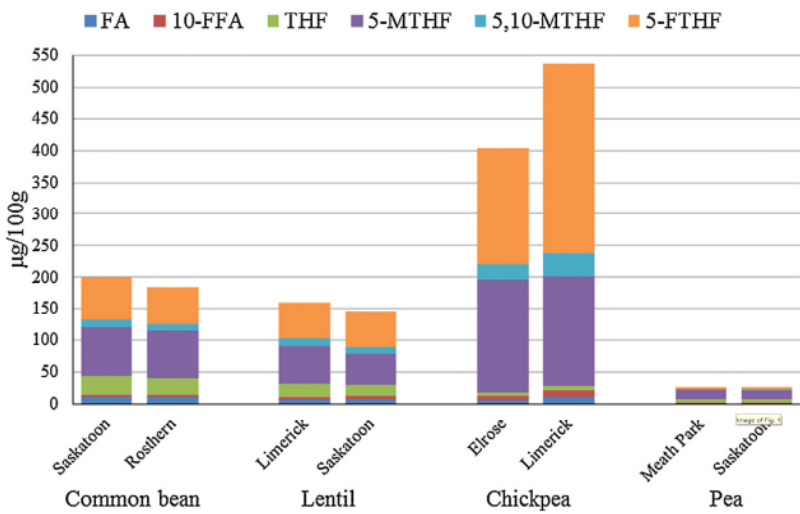
	Mg	Ca	Fe	Zn	Mn	Cu	Se
	µg/g						ng/g
Amit	1648	441 abc	51.7	27.1 bcd	22.9	6.6	731
CDC Cabri	1634	448 abc	55.0	26.4 abcd	22.3	6.7	636
CDC Corinne	1525	393 ab	48.6	24.4 abcd	25.5	7.1	712
CDC Frontier	1678	430 ab	54.1	21.1 ab	24.4	6.6	868
CDC Luna	1893	467 abc	52.2	21.2 abc	26.0	6.9	629
CDC Vanguard	1634	540 bcd	50.8	25.2 abcd	21.9	7.3	736
CDC Xena	1676	409 ab	49.1	27.7 cd	23.0	8.1	864
Myles	1902	644 cd	55.6	28.3 cd	25.4	8.7	677
Mean	1699	472	52.1	25.2	23.9	7.3	732

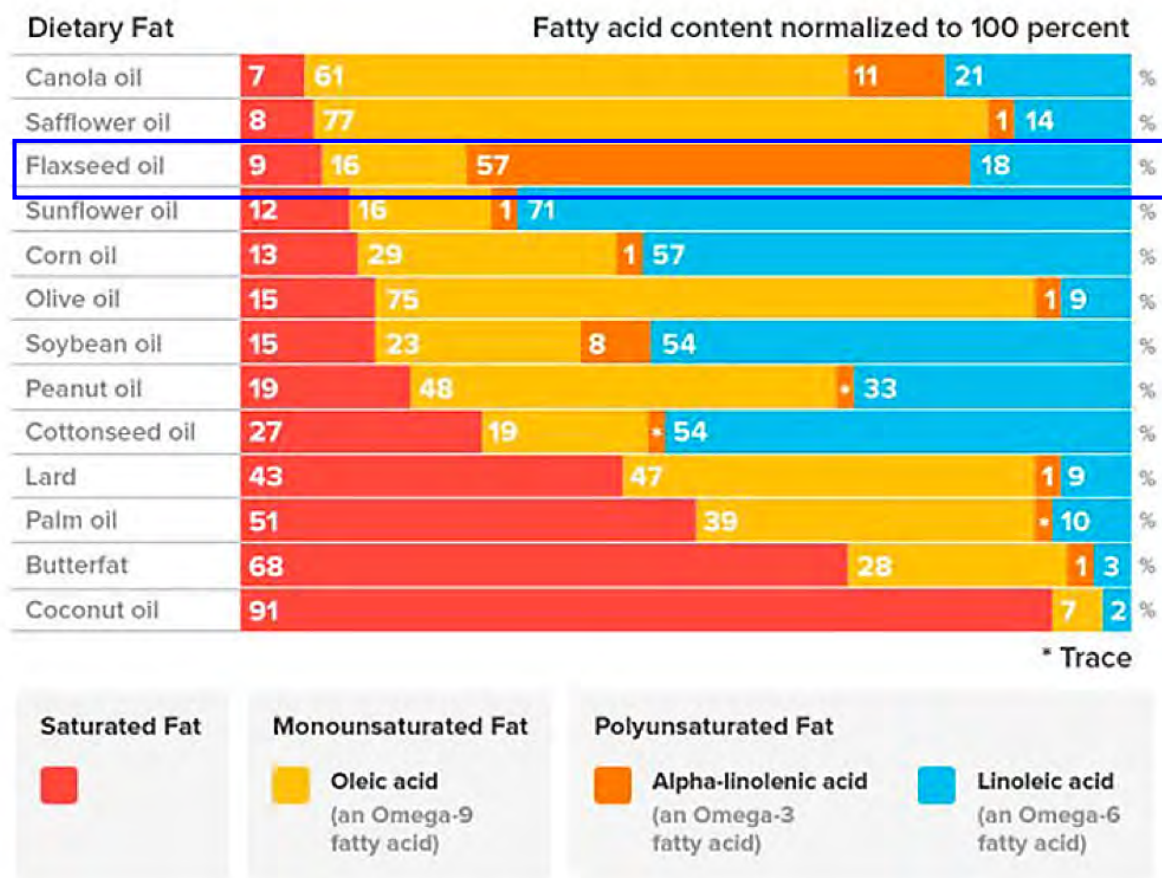
Total Carotenoids



Genetic diversity of folate profiles in seeds of common bean, lentil, chickpea and pea

Ambuj B. Jha^{a,1}, Kaliyaperumal Ashokkumar^{a,1}, Marwan Diapari^a, Stephen J. Ambrose^b, Haixia Zhang^b, Bunyamin Tar'an^a, Kirstin E. Bett^a, Albert Vandenberg^a, Thomas D. Warkentin^{a,*}, Randall W. Purves^{a,b}





Source: POS Pilot Plant Corporation

If chickpeas and flax are so super....

Why not
everyone eat
it?

Why do we not
produce them
in million tons?

What are the
challenges?

If chickpeas and flax are so super....

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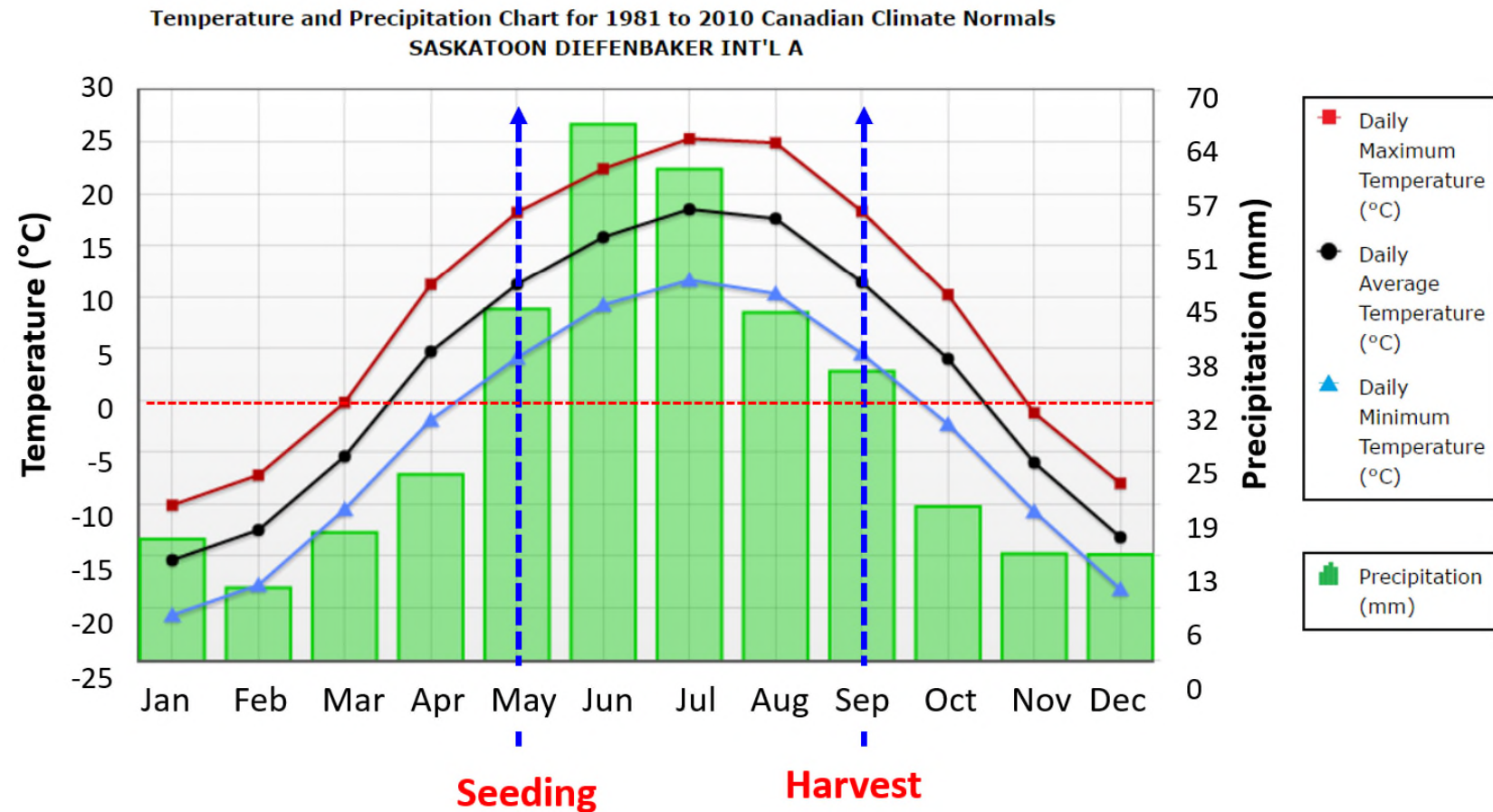
**What are the
challenges?**

Challenge #1: Short growing season in Saskatchewan

Average frost-free period: 110 days → chickpea matures in ~120 days

Seasonal cumulative DD_5 : 1274 (May – August) → Chickpeas= 1150-1350;

Mean annual precipitation: 368 mm



Challenge #2: High pressure of *Ascochyta* blight disease

- The most devastating disease globally caused by fungus *Ascochyta rabiei*
- Cool summer with some moisture in Saskatchewan → very conducive for the disease development.



Blight wipes out much of region's chickpea crops

By Richard Ripley
Staff writer

A chickpea blight epidemic has wiped out half of the Inland Empire crop of the popular yellowish salad bean, causing more than \$3 million in losses, the U.S. Department of

'It can wipe out a field in three days. It's just like fire.'

Challenge #3: Emerging Issue

PULSE ADVISOR

November 2020



Saskatchewan Chickpea Health Issue Report

Overview

A plant health issue was brought to the attention of Saskatchewan Pulse Growers (SPG) in late July of 2019 and occurred again in 2020. Samples from 2019 were evaluated by researchers Dr. Sabine Banniza from the Crop Development Centre (CDC) at the University of Saskatchewan and Dr. Michelle Hubbard from Agriculture and Agri-Food Canada (AAFC) Swift Current. Due to the late sampling in 2019 the samples all had a high level of *Ascochyta rabiei* that was insensitive to strobilurin fungicides, while no other foliar pathogens were identified. Some root samples did show signs of root rot with high prevalence of *Fusarium solani* and *Fusarium redolens* identified. In 2020, local growers and agronomists conducted extensive sampling on behalf of SPG for analysis of herbicide residues, nutrient levels, and foliar and root pathogens, performed at a commercial lab. Results from these tests have not identified any one cause of the chickpea health issue and it is likely that a combination of factors are involved. Further evaluations are currently underway.

Field Symptoms



Figure 2. Leaf chlorosis, tip die back, and whitening within the chickpea canopy (left). Wilting and chlorosis of leaflets of main and secondary

TOP CROP
MANAGER



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The mystery of the chickpea health issue

February 26, 2021

By Bruce Barker

Root rot? Herbicide carry-over? Insect damage? Nutrient deficiency?



Leaf tip chlorosis later in the season. Photos courtesy of Sherrilyn Phelps.

Challenge #3: 'New' Diseases!

frontiers | Frontiers in Plant Science

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Check for updates

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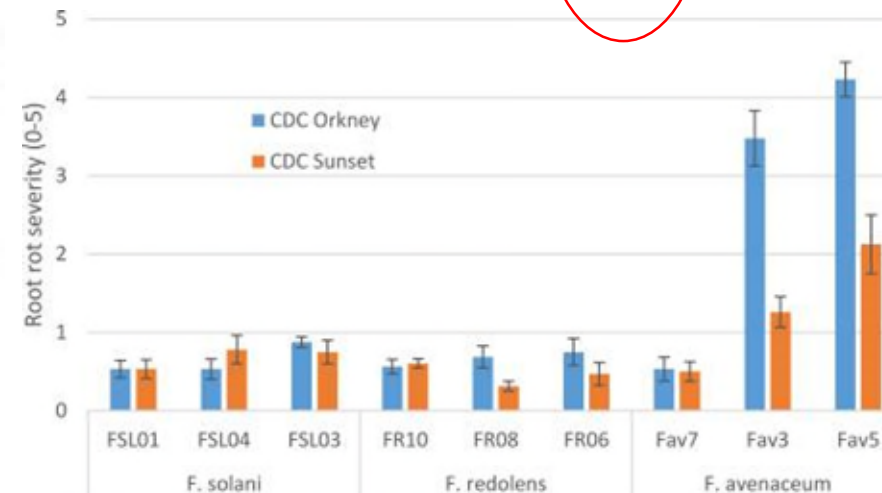
The chickpea root rot complex in Saskatchewan, Canada- detection of emerging pathogens and their relative pathogenicity

Cheryl Armstrong-Cho^{*†}, Nimlath Thangam Sivachandra Kumar[†],
Ramanpreet Kaur and Sabine Banniza

Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada



Root rot symptoms of CDC Leader kabuli chickpea under controlled conditions. From left to right: non-inoculated control, *Macrophomina phaseolina*, *Berkeleyomyces basicola*, *Verticillium dahliae*, ***Fusarium avenaceum***.



Root rot severity (0-5 scale) caused by three isolates each of three *Fusarium* species on 3-week-old plants

(Source: Armstrong-Cho et al. 2023)



Wheat



Canola



Flax



Chickpea

Challenge #4: acreage competition with other commodities, especially in a non-subsidized production and open market system

- Competitive advantage → increasing net return for farmers



Main goals of chickpea breeding program

1. Competitive advantage for farmers:

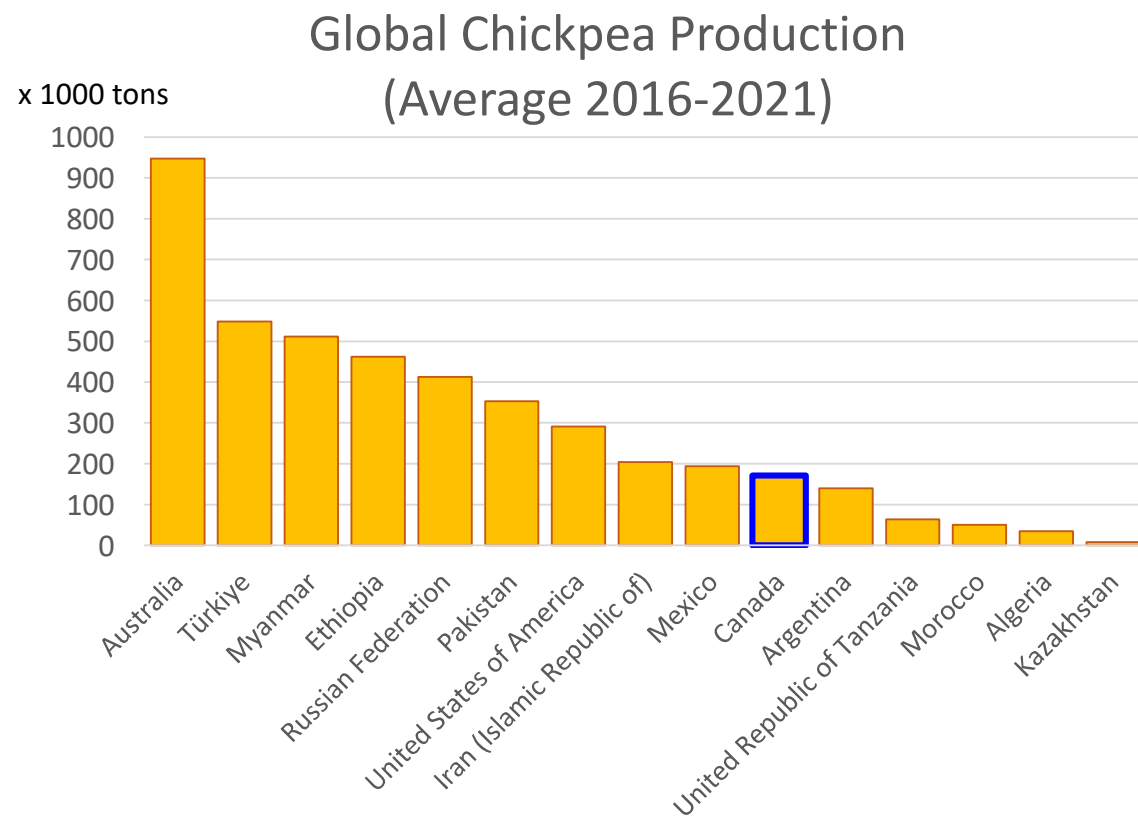
Yield x seed size (x nutritional quality) x price

2. **Reduce risks and production cost:** ascochyta blight resistance; fusarium root rot; early maturity; frost and heat tolerance

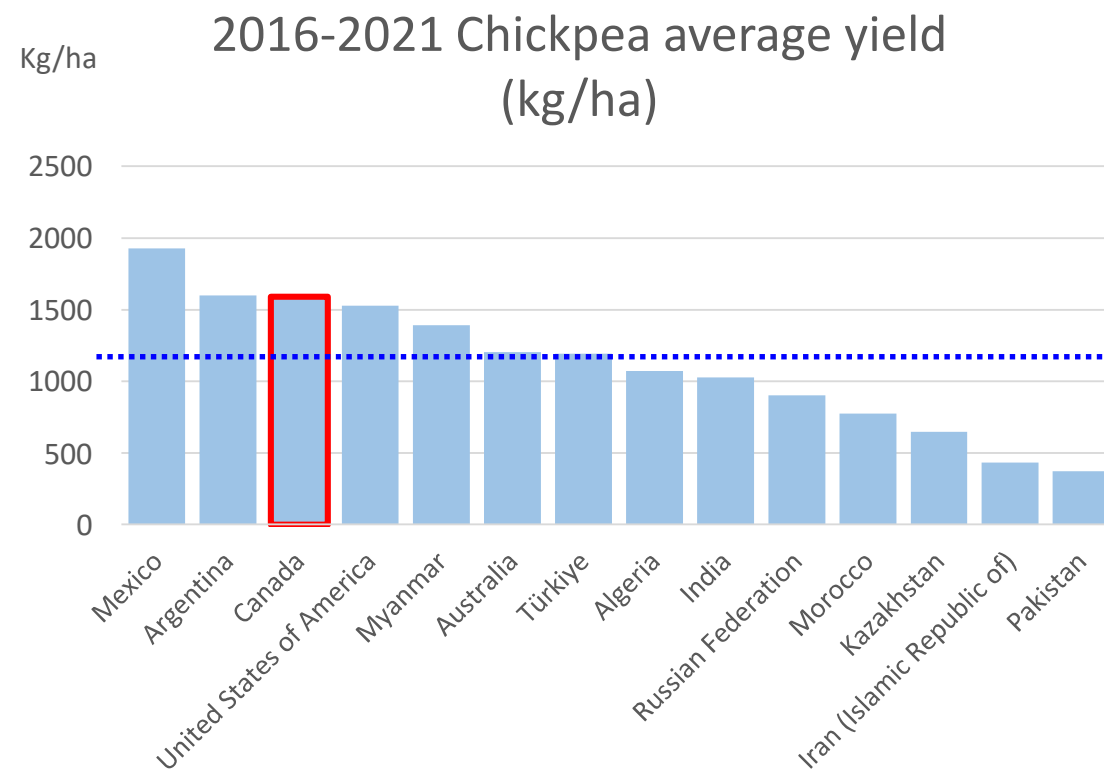
3. **Ease of management:** herbicide tolerance; upright plant stature

4. Consumer preference:

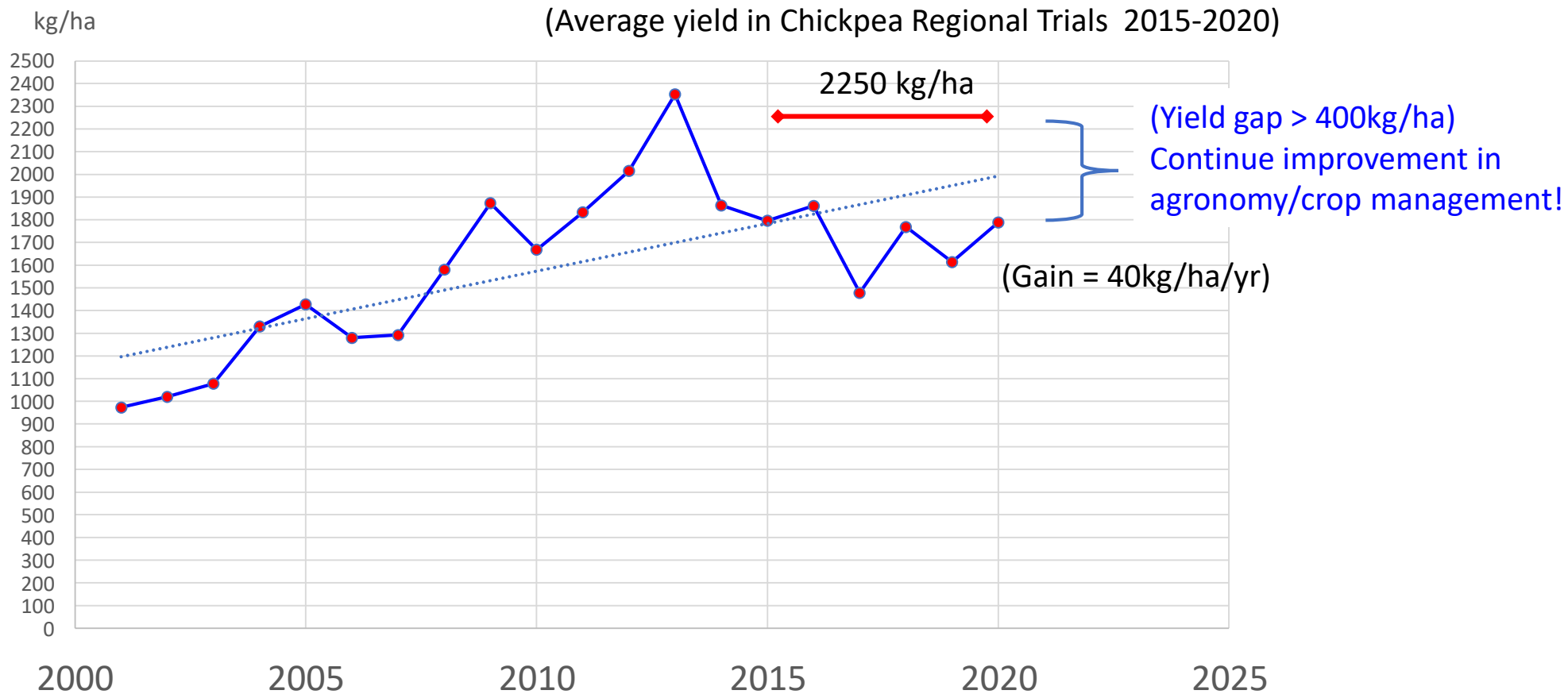
- Seed appearance (size, shape, colour);
- Grain processors: canning, milling efficiency;
- Nutritional characteristics (protein, oil, micronutrients, carotenoids);
- Alternative and new products: hummus, frozen green chickpea



(at the same period India produced: 10.1 M tons)



Source FAOSTAT 2023



Canada average chickpea yield (kg/ha) from 2001 to 2020

FAOSTAT 2023

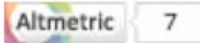
Early Maturity



The chickpea Early Flowering 1 (Efl1) locus is an ortholog of Arabidopsis ELF3

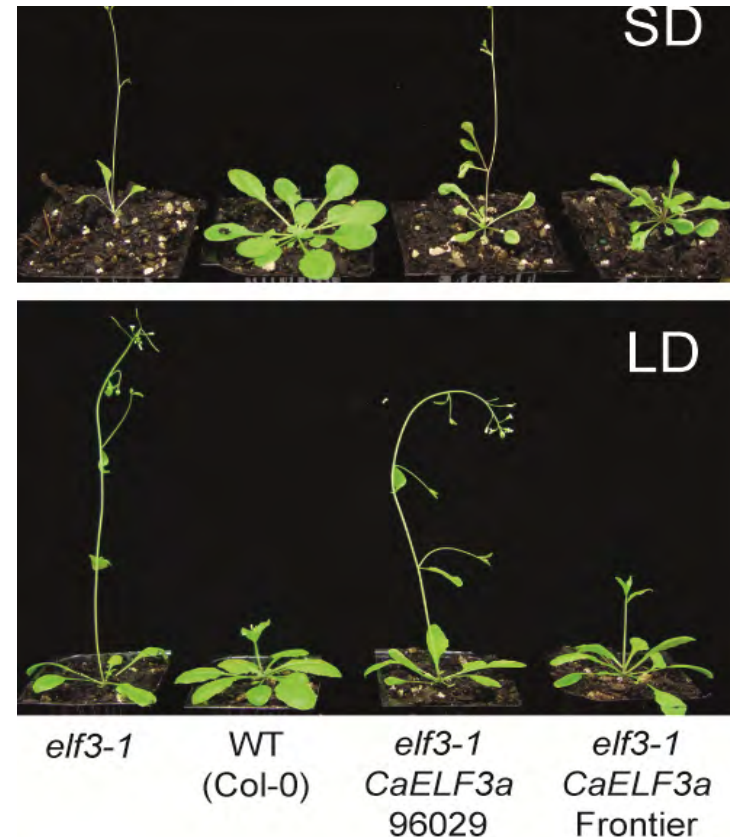
Stephen Ridge, Amit Deokar, Robyn Lee, Ketema Daba, Richard C. Macknight, James L. Weller, Bunyamin Tar'an

Published August 2017. DOI: <https://doi.org/10.1104/pp.17.00082>



Characterization of CaELF3 - key gene for early flowering

- Sequencing of ELF3 in ICCV 96029 and CDC Frontier: 11-bp deletion in highly conserved domain of ICCV 96029 resulting in a premature stop codon
- elf3* mutation strongly associated with reduced photoperiod sensitivity



- Transformation of *Arabidopsis elf3-1* mutant with CDC Frontier and ICCV 96029 forms

- Complementation of flowering phenotype of the *Arabidopsis elf3-1* mutant by the Frontier form, but not the 96029 form of 35S::CaELF3a

Early maturity strategy: *Early flowering; Less sensitive to photoperiod*
→ *3-5 days earlier maturity than the recurrent parent*

Introgression of early flowering *elf3* gene

ICCV96029 x CDC Frontier



F1 x CDC Frontier



BC1F1 x CDC Frontier



BC2F1 x CDC Frontier



BC3F1



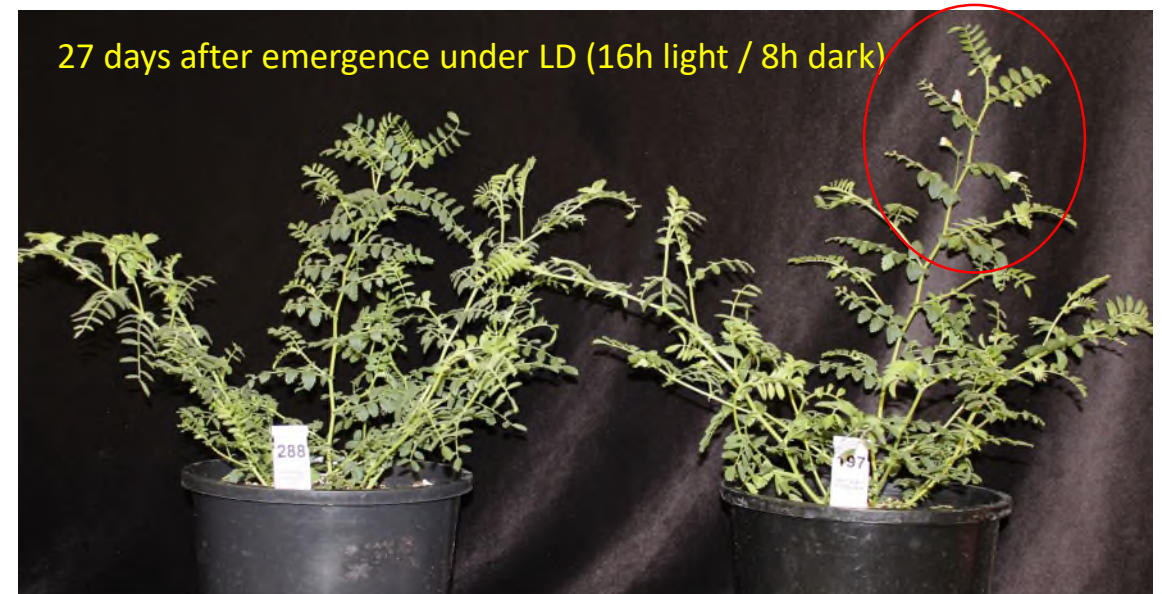
BC3F6

Foreground selection for *elf3*
& two flanking SNP markers

Foreground selection for *elf3*
& background selection with
40 markers (5 markers per chr)

Lines homozygous for *elf3* were selected

Phenotyping



DC Frontier (wild type/ELF3)

CDC Frontier with *elf3*
introgression

Resistance to fungal diseases:

- Description

- Resistance to ascochyta blight & potential new disease (s)

- Value Proposition

- Increased harvestable yield
- Stabilized yield
- Improved grain quality

- Approach

- Native resistances
- QTL, GWAS, Candidate genes
- Accelerated breeding strategy
- Gene editing and transgenic solutions (?)

Ascochyta blight nursery



Fusarium root rot



QTL sequencing strategy to map genomic regions associated with resistance to ascochyta blight in chickpea

Amit Deokar, Mandeep Sagi, Ketema Daba and Bunyamin Tar'an

Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Received 23 February 2018
 accepted 7 May 2018
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Keywords: ascochyta blight, chickpea, NGS-based BSA, QTL, genomics, sequencing.

Introduction

Ascochyta blight caused by the necrotrophic fungus *Ascochyta blight* (CDB) is one of the most devastating diseases of chickpea (*Cicer arietinum* L.) worldwide. The disease frequently occurs with high severity in areas with cool and wet growing conditions such as Canada, United States and some parts of Mediterranean countries (Sharma and Gnan, 2016). Under favourable conditions, ascochyta blight can infect chickpea plants at any growth stage. However, the crop is more susceptible at flowering and podding stages causing substantial economic damage to the crop (Sharma et al., 2013). A significant decline in chickpea production in Canada and Australia in the past decade was resulted from heavy yield losses caused by ascochyta blight (Armstrong-Cho et al., 2008; Bretag et al., 2008). At present, successful chickpea production in many areas depends on effective ascochyta blight management. Genotypes with complete resistance to ascochyta blight in chickpea are lacking. However, moderately resistant genotypes have been identified and used to develop cultivars with improved resistance. In areas where ascochyta blight infection is predominant, these cultivars were used along with fungicide applications to manage the disease. This strategy, however, is often ineffective when the conditions for ascochyta blight infection are highly conducive. Therefore, continuing efforts to develop new cultivars with improved resistance to ascochyta blight is required to sustain chickpea production.

Resistance to ascochyta blight is polygenic, and is often highly affected by environmental conditions (Armstrong-Cho et al.,

Summary
 Whole-genome sequencing-based bulked segregant analysis (BSA) for mapping quantitative trait loci (QTL) provides an efficient alternative approach to conventional QTL analysis as it significantly reduces the scale and cost of analysis with comparable power to QTL detection using full mapping population. We tested the application of next-generation sequencing (NGS)-based BSA approach for mapping QTLs for ascochyta blight resistance in chickpea using two recombinant inbred line populations CBR-01 and CBR-02. Eleven QTLs in CBR-01 and six QTLs in CBR-02 populations were mapped on chromosomes Ca1, Ca2, Ca4, Ca6 and Ca7. The QTLs identified in CBR-01 using conventional biparental mapping approach were used to compare the efficiency of NGS-based BSA in detecting QTLs for ascochyta blight resistance. The QTLs on chromosomes Ca1, Ca4, Ca6 and Ca7 overlapped with the QTLs previously detected in CBR-01 using conventional QTL mapping method. The QTLs on chromosome Ca4 were detected in both populations and overlapped with the previously reported QTLs including unmarked region for ascochyta blight resistance across different chickpea genotypes. Six candidate genes in the QTL regions identified using NGS-based BSA on chromosomes Ca2 and Ca4 were validated for their association with ascochyta blight resistance in the CBR-02 population. This study demonstrated the efficiency of NGS-based BSA as a rapid and cost-effective method to identify QTLs associated with ascochyta blight in chickpea.

ARTICLE

QTL mapping of early flowering and resistance to ascochyta blight in chickpea

Ketema Daba, Amit Deokar, Sabine Banniza, Thomas D. Warkentin, and Bunyamin Tar'an

Abstract: In western Canada, chickpea (*Cicer arietinum* L.) production is challenged by short growing seasons and infestations with ascochyta blight. Research was conducted to determine the genetic basis of the association between flowering time and reaction to ascochyta blight in chickpea. Ninety-two chickpea recombinant inbred lines (RILs) developed from a cross between ICV 96029 and CDC Frontier were evaluated for flowering responses and ascochyta blight reactions in growth chambers and fields at multiple locations and during several years. A wide range of variation was exhibited by the RILs for days to flower, days to maturity, node of first flowering, plant height, and ascochyta blight resistance. Moderate to high broad-sense heritability was estimated for ascochyta blight reaction ($H^2 = 0.24$ – 0.34) and for days to flowering ($H^2 = 0.45$ – 0.87) depending on the environments. Negative correlations were observed among the RILs for days to flowering and ascochyta blight resistance, ranging from $r = -0.21$ ($P < 0.05$) to -0.58 ($P < 0.0001$). A genetic linkage map consisting of eight linkage groups was developed using 349 SNP markers. Seven QTLs for days to flowering were identified that individually explained 9%–44% of the phenotypic variation. Eight QTLs were identified for ascochyta blight resistance that explained phenotypic variation ranging from 10% to 19%. Clusters of QTLs for days to flowering and ascochyta blight resistance were found on chromosome 3 at the interval of 8.6–23.1 cM and on chromosome 8 at the interval of 53.88–62.33 cM.

Key words: *Cicer arietinum*, *Ascochyta blight*, early flowering, QTLs.

Résumé: Au Canada, la production de pois chiches (*Cicer arietinum* L.) est confrontée à une saison de croissance courte et à l'ascochyta blight. Des travaux de recherche ont été réalisés pour élucider l'association génétique de l'association entre la date de floraison et la réaction à l'ascochyta blight dans le pois chiche. Quarante-deux lignées RIL développées à partir d'un croisement entre ICV 96029 et CDC Frontier ont été évaluées pour la date de floraison et la réaction à l'ascochyta blight dans des chambres de croissance et en champs. Une large gamme de variation a été observée pour les caractères suivants : la date de floraison, la date de maturité, le premier nœud porteur de fleurs, la taille des plantes et la résistance à l'ascochyta blight. Une héritabilité modérée à élevée a été observée pour la résistance à l'ascochyta blight ($H^2 = 0.24$ à 0.34) et pour la date de floraison ($H^2 = 0.45$ à 0.87), selon l'environnement. Des corrélations négatives ont été observées entre la date de floraison et la résistance à l'ascochyta blight. Une carte génétique comprenant huit groupes de liaison a été produite à l'aide de 349 marqueurs SNP. Sept QTLs pour la date de floraison ont été identifiés ; chacun expliquant individuellement entre 9 % et 44 % de la variation phénotypique. Huit QTLs ont été identifiés pour la résistance à l'ascochyta blight et chacun expliquant entre 10 % et 19 % de la variation phénotypique. Des groupements de QTLs pour la date de floraison et la résistance à l'ascochyta blight ont été notés sur les chromosomes 3 et 8. (Traduit par la Rédaction)

Meta-ids: *Cicer arietinum*, *Ascochyta blight*, floraison précoce, QTL.

Introduction

Chickpea (*Cicer arietinum* L., $2n = 2x = 16$) is one of the most important food legumes in the world used to dry bean (FAOSTAT 2013). The crop was recently introduced to Canada, and since then, the area under chickpea production in Canada has been fluctuating. Flowering time and maturity

are among the important characteristics of chickpea that are critical for the adaptation of chickpea cultivars world wide (Arboreli et al., 2006; Upadhyay et al., 2005; Sharma and Ghosh 2016). Ascochyta blight caused by the fungus *Ascochyta blight* Pao. Lab. has emerged as one of the most destructive diseases of the crop globally (Stephens et al.,

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ORIGINAL ARTICLE

Genome-wide SNP discovery for development of high-density genetic map and QTL mapping of ascochyta blight resistance in chickpea (*Cicer arietinum* L.)

Amit Deokar¹, Mandeep Sagi¹, Bunyamin Tar'an¹

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 © The Author(s) 2019

Abstract

Key message: A high-density linkage map of chickpea using 3430 SNPs was constructed and used to identify QTLs and candidate genes for ascochyta blight resistance in chickpea.

Abstract: Chickpea cultivation in temperate conditions is highly vulnerable to ascochyta blight infection. Cultivation of resistant cultivars in combination with fungicide application within an informed disease management package is the most effective method to control ascochyta blight in chickpea. Identifying new sources of resistance is critical for continued improvement in ascochyta blight resistance in chickpea. The objective of this study was to identify genetic loci and candidate genes controlling the resistance to ascochyta blight in recombinant inbred lines derived from crossing cultivars Amit and ICV 96029. The RILs were genotyped using the genotyping-by-sequencing procedure and Illumina[®] GoldenGate array. The RILs were evaluated in the field over three site-years and in three independent greenhouse experiments. A genetic map with eight linkage groups was constructed using 3430 SNPs. Eight QTLs for resistance were identified on chromosomes 2, 3, 4, 5 and 6. The QTLs individually explained 7–40% of the phenotypic variations. The QTLs on chromosomes 2 and 6 were associated with the resistance at vegetative stage only. The QTLs on chromosomes 2 and 4 that were previously reported to be conserved across diverse genetic backgrounds and against different isolates of *Ascochyta blight* were confirmed in this study. Candidate genes were identified within the QTL regions. Their co-localization with the underlying QTLs was confirmed by genetic mapping. The candidate gene-based SNP markers would lead to more efficient marker-assisted selection for ascochyta blight resistance and would provide a framework for fine mapping and subsequent cloning of the genes associated with the resistance.

Introduction

Chickpea (*Cicer arietinum* L.) is the world's second most important grain legume. Multiple pests and diseases significantly affect chickpea productivity. Among the diseases, ascochyta blight caused by the necrotrophic fungal pathogen

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Genetic Analysis of NBS-LRR Gene Family in Chickpea and Their Expression Profiles in Response to Ascochyta Blight Infection

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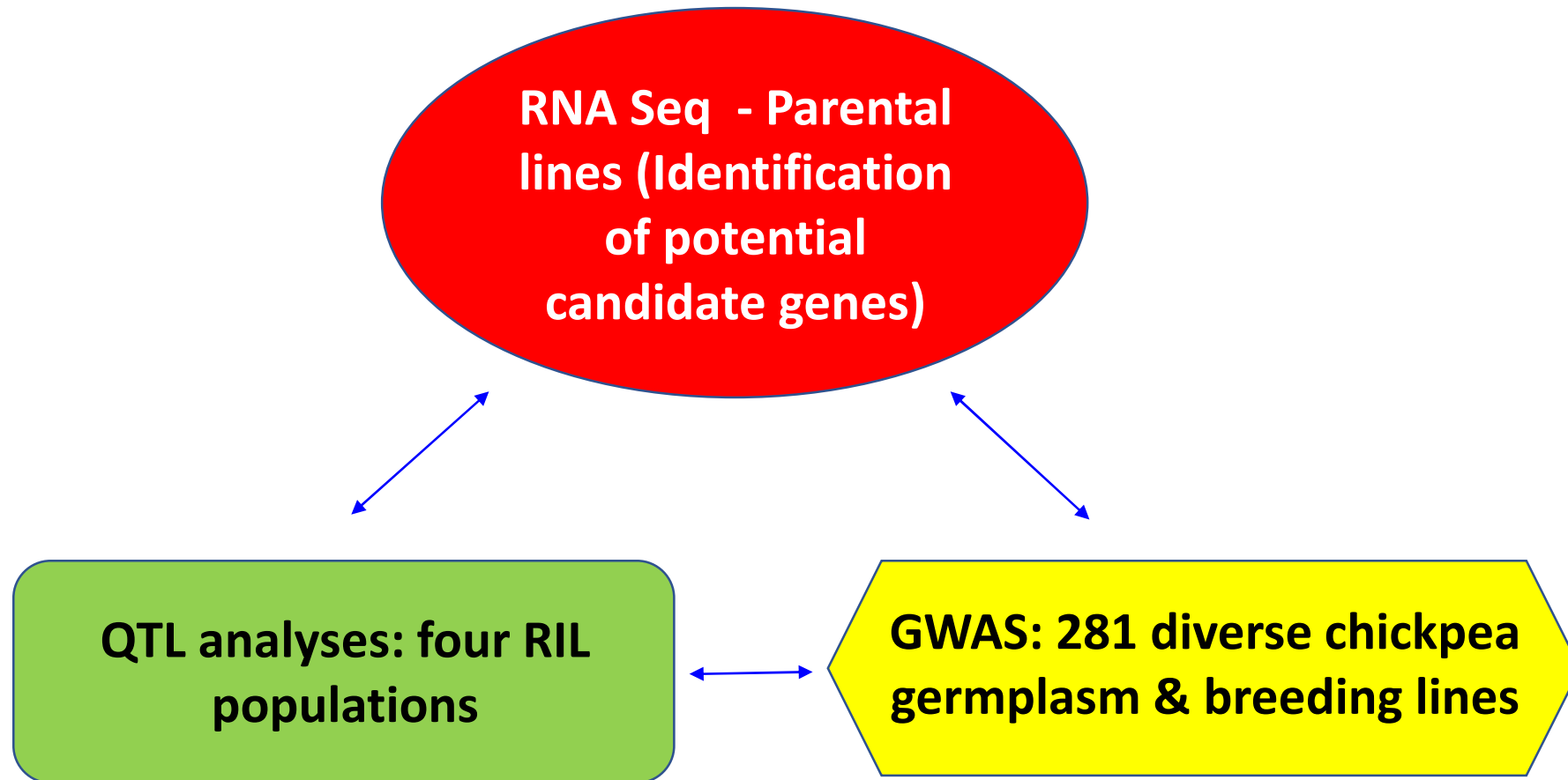
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 Sagi MS, Deokar AA and Tar'an B (2019) Genetic Analysis of NBS-LRR Gene Family in Chickpea and Their Expression Profiles in Response to Ascochyta Blight Infection. Front. Plant Sci. 10:4324. doi: 10.3389/fpls.2019.00432

Ascochyta blight is one of the major diseases of chickpea worldwide. The genetic resistance to ascochyta blight in chickpea is complex and governed by multiple QTLs. However, the molecular mechanism of quantitative disease resistance to ascochyta blight and the genes underlying these QTLs are still unknown. Most often disease resistance is determined by resistance (R) genes. The most predominant R-genes contain nucleotide binding site and leucine rich repeat (NBS-LRR) domains. A total of 121 NBS-LRR genes were identified in the chickpea genome. Ninety-eight of these genes contained all essential conserved domains while 23 genes were truncated. The NBS-LRR genes were grouped into eight distinct classes based on their domain architecture. Phylogenetic analysis grouped these genes into two major clusters based on their structural variation, the first cluster with toll or interleukin-1 like receptor (TIR) domain and the second cluster either with or without a coiled-coil domain. The NBS-LRR genes are distributed unevenly across the eight chickpea chromosomes and nearly 50% of the genes are present in clusters. Thirty of the NBS-LRR genes were co-localized with nine of the previously reported ascochyta blight QTLs and were tested as potential candidate genes for ascochyta blight resistance. Expression pattern of these genes was studied in two resistant (CDC Corinne and CDC Luna) and one susceptible (ICV 96029) genotypes at different time points after ascochyta blight infection using real-time quantitative PCR. Twenty-seven NBS-LRR genes showed differential expression in response to ascochyta blight infection in at least one genotype at one time point. Among these 27 genes, the majority of the NBS-LRR genes showed differential expression after inoculation in both resistant and susceptible genotypes which indicates the involvement of these genes in response to ascochyta blight infection. Five NBS-LRR genes showed genotype specific expression. Our study provides a new insight of NBS-LRR gene family in chickpea and the potential involvement of NBS-LRR genes in response to ascochyta blight infection.

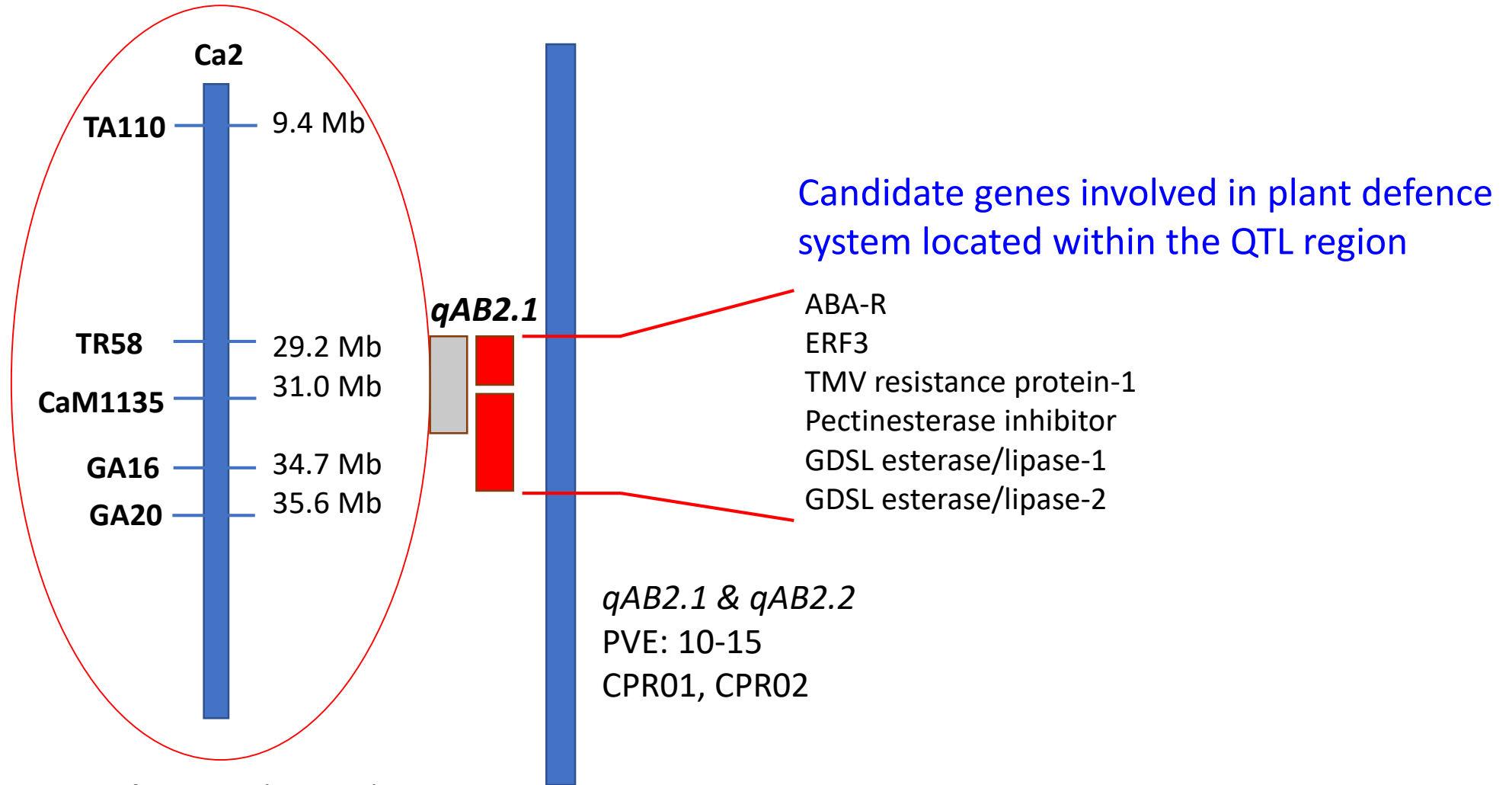
Keywords: NBS-LRR genes, expression profiling, ascochyta blight, chickpea

QTLs for resistance to ascochyta blight (in order of importance): Chr4, Chr2, Chr3, Chr5, Chr 8

Identification of potential candidate genes for resistance to ascochyta blight using RNA-Seq, QTL analyses and GWAS.

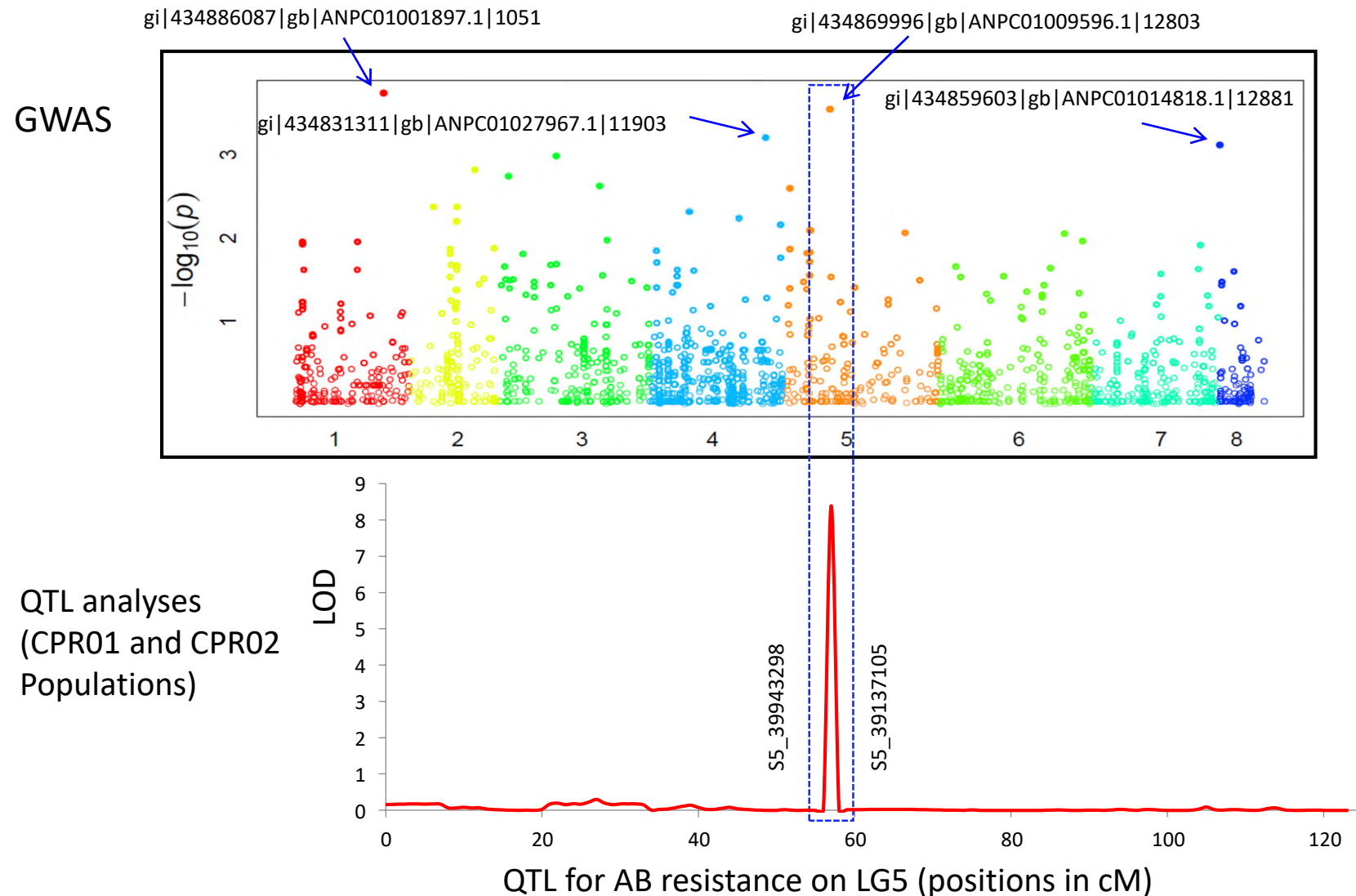


Conserved QTL across populations



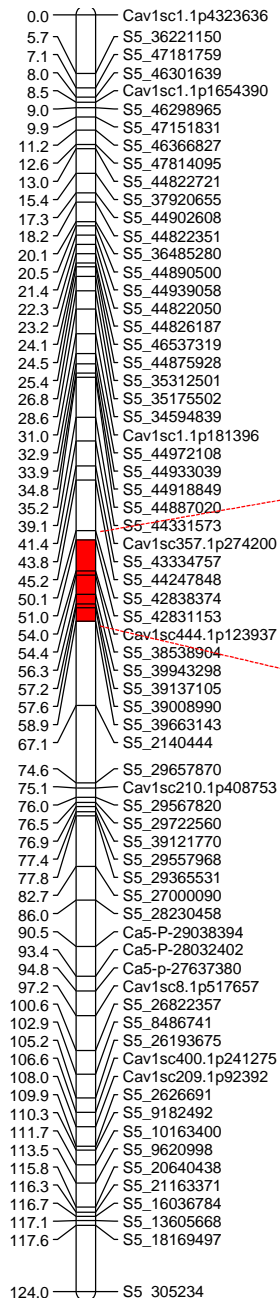
ara1* and *ara2a Udupa and Baum 2003,
Thudi et al. 2011, QTLAR3 (Iruela et al 2007)
ABQTL-1 (Varshney et al 2013),
Ar19 (Cho et al 2004)

Identification of genomic region associated with AB using genome wide association analysis and bi-parental mapping populations (QTL analysis)



Integrating transcript profiles with QTL mapping

Genes located in QTL interval and differentially expressed in response to AB infections were selected as potential candidate genes

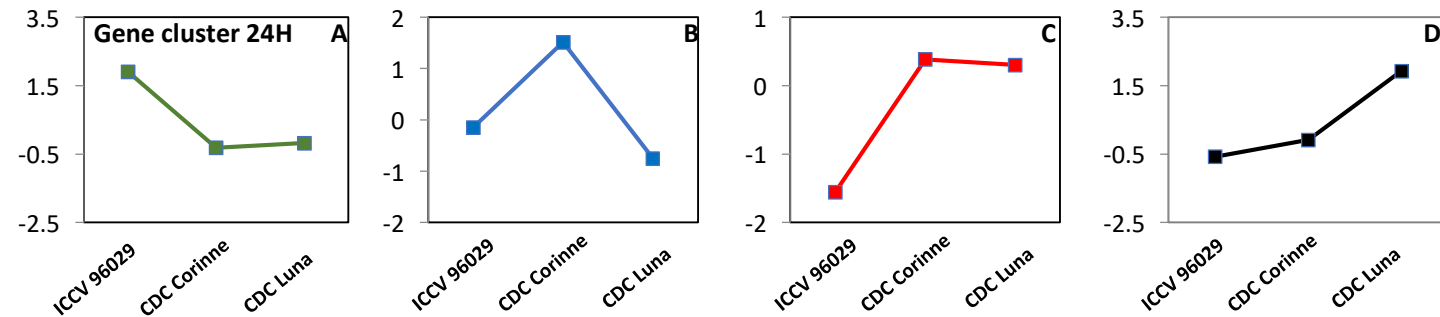


Physical map
CDC Frontier CaV1.0
Ca5

QTL for AB

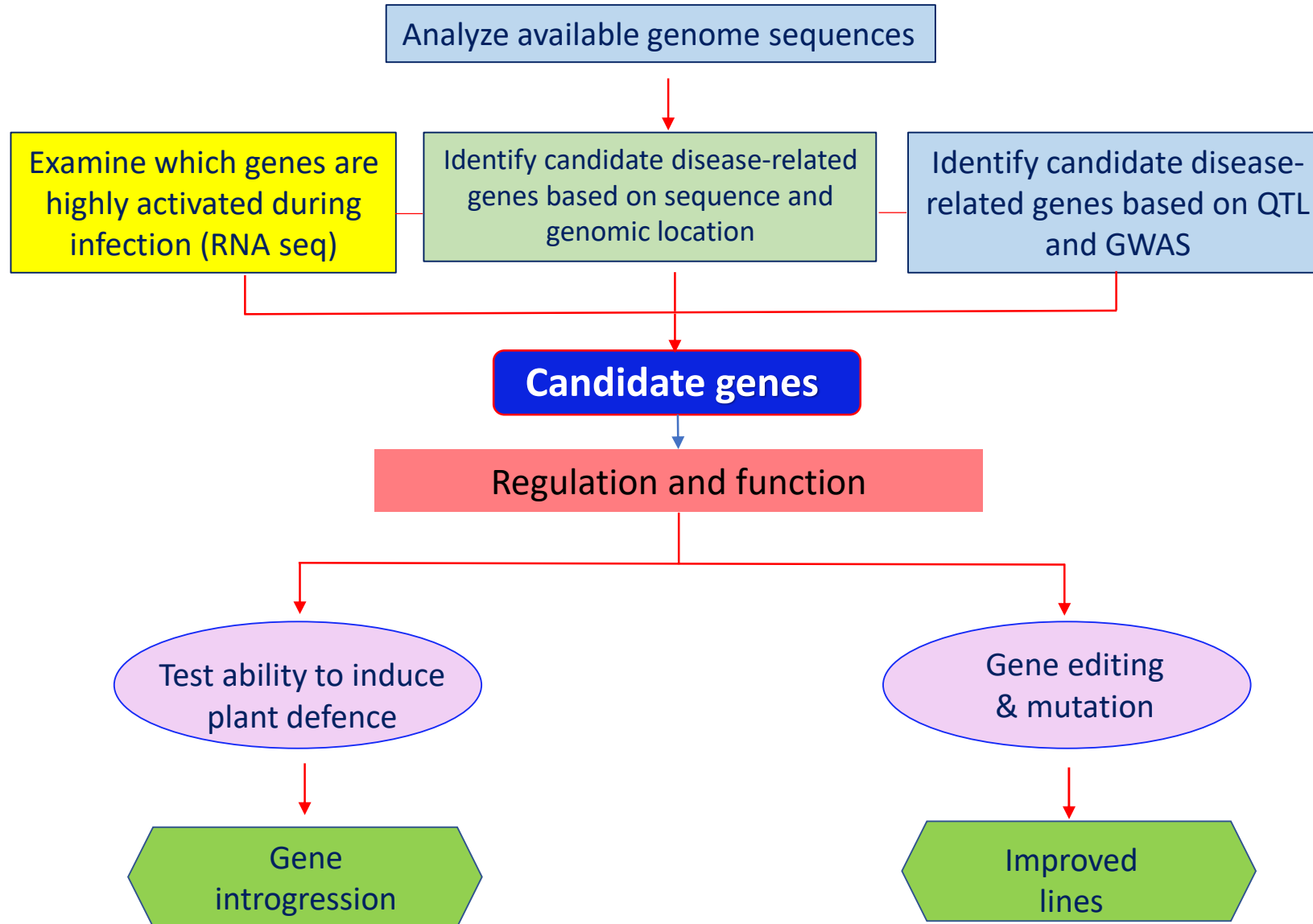
Field and green house
screening
LOD= 4 - 17
PVE (%) = 12-46%

Time	Gene Cluster	Gene ID	Annotation
24H	B	Ca_11396	adenosylhomocysteinase
24H	C	Ca_07411	callose synthase 10
24H	C	Ca_07641	zinc finger protein CONSTANS 9
24H	D	Ca_03876	amino acid permease 3
48H	A	Ca_07425	abhydrolase domain-containing protein
48H	A	Ca_07560	probable serine/threonine-protein kinase
48H	A	Ca_07602	beta-carotene isomerase D27, chloroplastic
48H	A	Ca_11311	small heat shock protein, chloroplastic
48H	A	Ca_12654	aldehyde dehydrogenase
48H	B	Ca_07508	alpha,alpha-trehalose-phosphate synthase
48H	B	Ca_12643	beta-galactosidase 16
48H	B	Ca_12669	gamma-glutamyltranspeptidase 2
48H	D	Ca_07629	repetitive proline-rich cell wall protein 2
72 H	A	Ca_07615	CYSTM1 family protein A
72 H	A	Ca_11408	chalcone synthase 1B



Expression pattern of candidate genes in ICCV 96029, CDC Corinne and CDC Luna

Genomic approach to control Ascochyta blight in chickpea



Identifying New Sources of Resistance to Ascochyta Blight in Chickpea

Elizabeth Berenik, Tamanna Jahan, Shweta Kalve, and Bunyamin Tar'an
Crop Development Center/Department of Plant Sciences, University of Saskatchewan

Introduction

Ascochyta blight, caused by the fungus *Ascochyta rabiei* Pass. Lab. is a detrimental disease to chickpea (*Cicer arietinum* L.) causing yield losses of up to 100% under conducive environments (Navas-Cortés et al., 1998). With the emergence of more aggressive fungal populations and varying climatic conditions, stronger and more durable resistance to Ascochyta blight in chickpeas is needed. An abundance of genetic variability is present within the wild chickpea species, including resistance to Ascochyta blight. Previous research has shown strong resistance to Ascochyta blight is present in *Cicer judaicum*, however, no fertile progeny has been recovered from crossing *C. judaicum* and cultivated chickpea. *Cicer pinnatifidum* has successfully been crossed with cultivated species and is being used as a potential bridge species (Mallikarjuna and Jadhav, 2008). An interspecific population consisting of 200 F₆ lines derived from a cross between *C. pinnatifidum* x *C. judaicum* were screened for their reaction to Ascochyta blight under field and greenhouse conditions.

Materials and Methods

A total of 200 lines were developed from an interspecific cross of *Cicer pinnatifidum* x *Cicer judaicum*. These lines were increased in the greenhouse to F₆ and screened for resistance to Ascochyta blight. ICCV 96029, as a highly susceptible cultivar was used control. Eight CDC chickpea commercial cultivars were used as resistant checks. All plant materials were planted in the greenhouse and inoculated with *A. rabiei* isolate AR170-03. Disease progression was rated based on a the Horsfall and Barratt 1- 12 rating scale at 7, 14, and 21 days after inoculation. The plant material is being subjected to disease screening with 2 repeats, each containing 4 replications.

Results

A frequency distribution at 14 days post inoculation (Figure 1) shows the range of disease scores within the lines. This range of scores is significantly different than that obtained from the control plants ($p = 0.04$). Based on a best linear unbiased predictor test, seven lines were deemed as highly resistant through all three rating periods (Figure 2). Based on a box and whisker test (Figure 3) we see that the lines have a wide range of distribution, out competing that of the controls. A closer look of F₆ line 62 (Figure 4) we see a slower progression of disease than that of the control included in its pot.

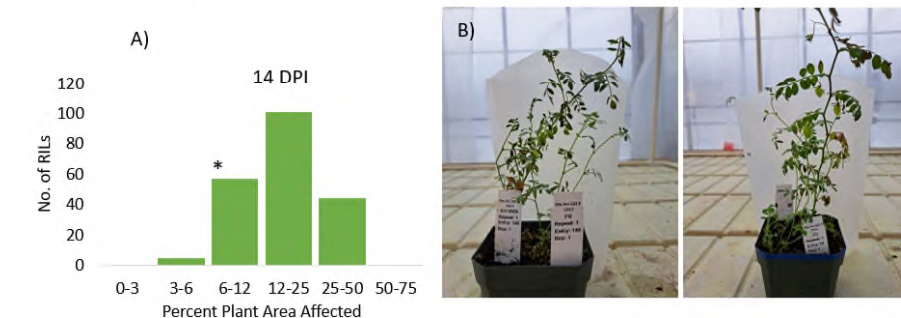


Figure 1. A) Frequency distribution of the mean ratings at 14 days post inoculation under controlled conditions. * indicates the scores of the parental lines. B) Selected F₆ lines (RILs # 145 and 62) with control ICCV96029 for use in crossing block as donor for new resistance to ascochyta blight.

References

Mallikarjuna, N., and Jadhav, D.R. (2008) Techniques to produce hybrid between *Cicer arietinum* L. x *C. pinnatifidum* Jaub. Indian Journal of Genetics and Plant Breeding, 68 (4), pp. 398-405.
Navas-Cortés JA, Pérez-Artés E, Jiménez-Díaz RM, Liobell A, Bainbridge BW, Heale JB, 1998. Mating type, pathotype, and RAPDs analysis in *Didymella rabiei*, the agent of ascochyta blight of chickpea. *Phytoparasitica* 26, 199– 212.

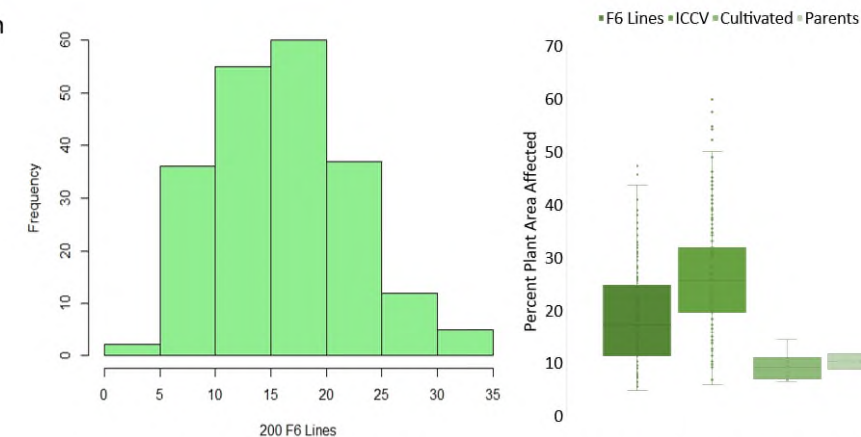


Figure 2. A histogram containing the results of a best linear unbiased predictor test over the three rating periods.

Figure 3. Distributions of the 200 F₆ lines, ICCV 96029, CDC varieties and parental lines.

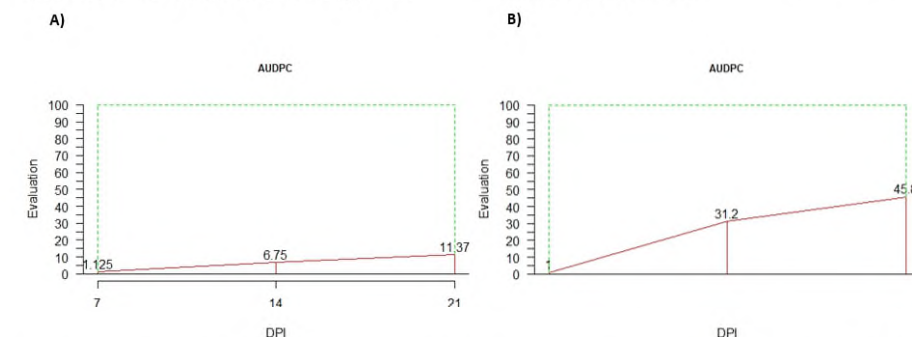


Figure 4. An area under the disease progression curve depicting ascochyta blight development in the greenhouse at 7, 14, and 21 days after inoculation (DPI). A) represents F₆ line 62 B) represents the control included in the pot with line 62.

Conclusions and Future Research

The current greenhouse screening has identified multiple F₆ lines with strong resistance to ascochyta blight. Few lines have resistance levels higher than the parental lines indicating that *Cicer pinnatifidum* and *Cicer judaicum* parents carry different genes for resistance. Selected resistance lines are being used as donor in crossing block to introgress the resistance into cultivated chickpea. To further confirm this resistance, additional screening in the greenhouse and field is needed. Linkage map development and quantitative trait loci (QTLs) analysis will be conducted to identify genomic regions corresponding with the resistance.

Acknowledgments:

CDC Pulse Crop Breeding Staff
CDC Pulse Pathology Lab
Robert P. Knowles Scholarship



Government
of
Saskatchewan

WGRF
Advancing Agriculture through Research

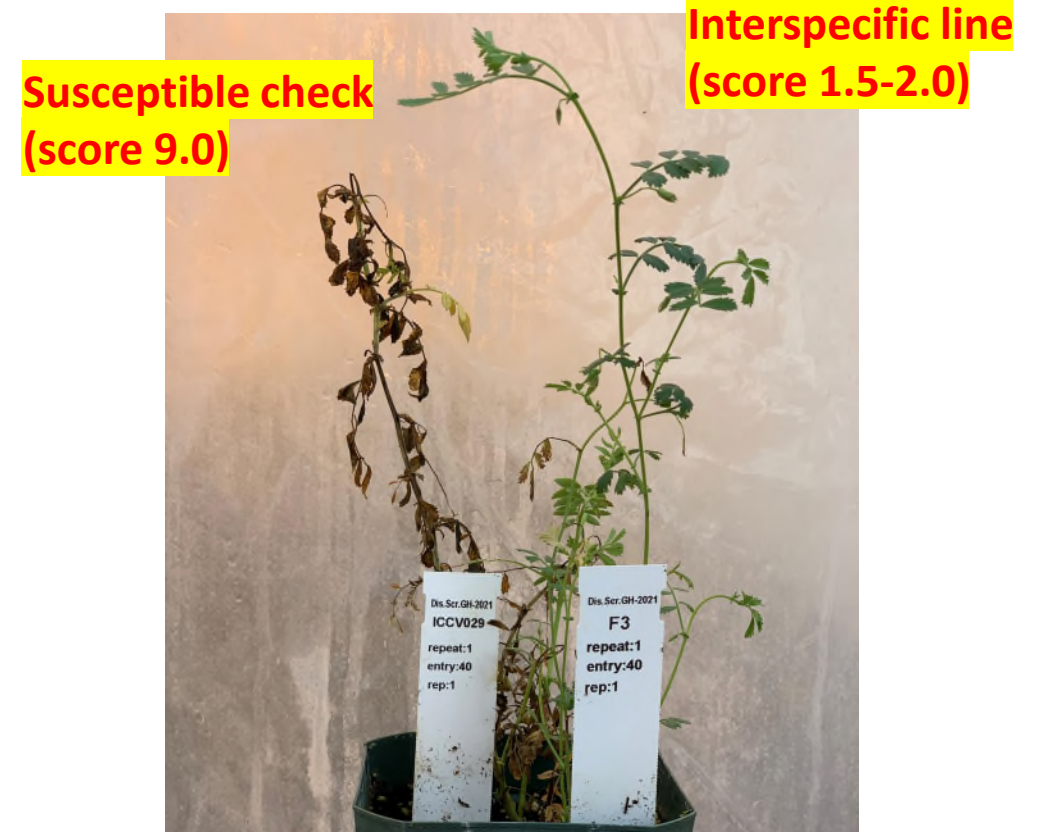
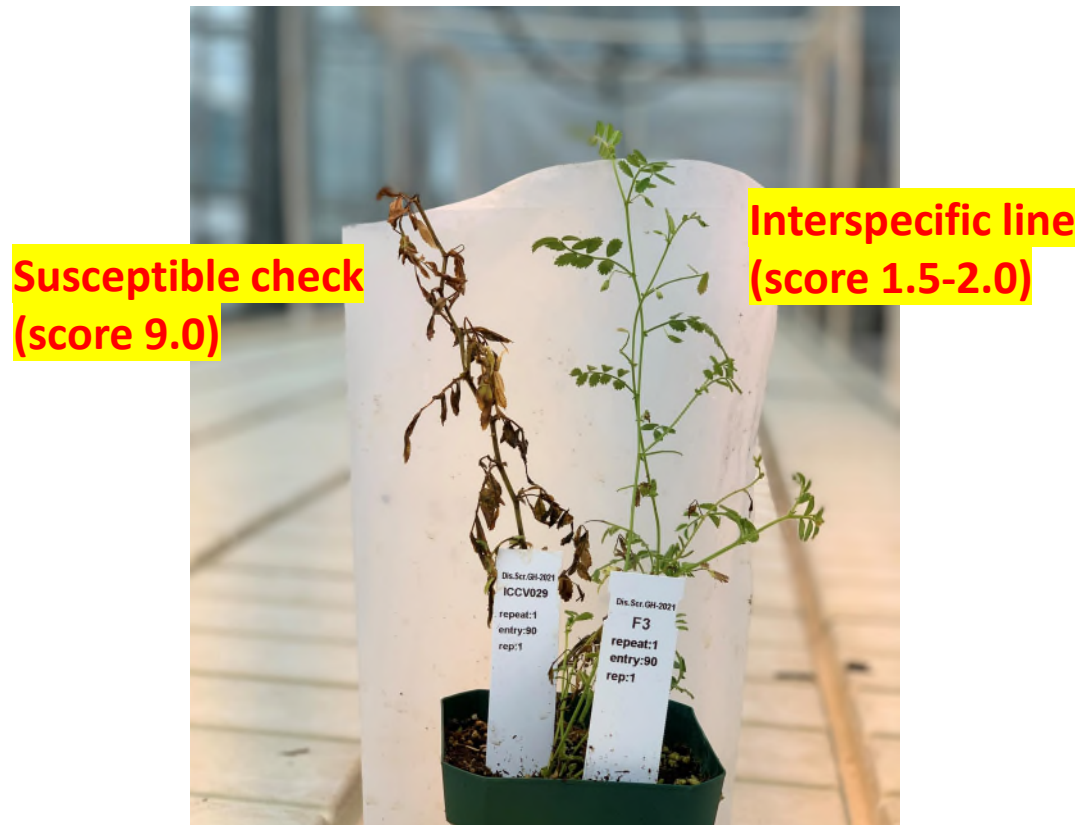
Elizabeth Berenik
MSc student

Cicer arietinum

C. pinnatifidum

C. judaicum

ADF #20200134 Diversifying Sources for Resistance to Ascochyta Blight in Chickpea
(Matching fund: WGRF)



2023 Field evaluation: Moose Jaw, Elrose, Saskatoon (irrigation)

Screening for Ascochyta blight resistance of the progeny from interspecific cross
between *Cicer pinnatifidum* and *C. judaicum*

Abiotic Stress Tolerance

“Mining” wild relatives for tolerance to abiotic stresses

C. arietinum x *C. reticulatum* // *C. arietinum* x *C. reticulatum*
(CDC Leader) (19 different accessions)

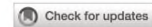
600 lines

F₅ (200 lines)

selected for seed quality and resistance
to Ascochyta Blight

Selfing until F₈

(200 interspecific lines)



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Genome-wide association analysis of stress tolerance indices in an interspecific population of chickpea

Shweta Kalve*, Krishna Kishore Gali and Bunyamin Tar'an

Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Chickpea is a cool season crop that is highly vulnerable to abiotic stresses such as heat and drought. High temperature during early flowering and pod development stages significantly reduces the crop yield. The wild relatives of chickpeas can be potential donors for the introgression of heat and drought tolerance into cultivated chickpeas for crop improvement. Initially, 600 interspecific lines were derived from crosses between two elite cultivars, CDC Leader (kabuli chickpea) and CDC Consul (desi chickpea), and 20 accessions of *Cicer reticulatum*. The F₅ interspecific lines were tested for agronomic and seed quality traits including reaction to ascochyta blight disease under field conditions at two locations in 2018. A subset of 195 lines were selected based on resistance to ascochyta blight and acceptable seed quality. These lines were evaluated for their performance under suboptimal conditions at Lucky Lake (2019 and 2020) and Moose Jaw (2019), Saskatchewan, Canada, and Yuma, Arizona, United States (2019–2020). The lines were grown and evaluated at two seeding dates, normal (SD1) and late (SD2) seeding dates, at each location and year. The same lines were genotyped using Cicer60K Axiom® SNP chip. The population structure was determined based on 35,431 informative SNPs using fastStructure, and the interspecific lines were clustered at a *k*-value of 15. Significant marker-trait associations were identified for seed yield from SD1 and SD2 seeding dates, and stress tolerance indices (ATI, K₁STI, MP, SSPI, and TOL) using phenotypic values both from individual locations and combined analyses based on BLUP values. SNP marker Ca2_34600347 was significantly associated with yield from both the seeding dates. This and other SNP markers identified in this study may be useful for marker-assisted introgression of abiotic stress tolerance in chickpea.

KEYWORDS

suboptimal conditions, interspecific crosses, marker-assisted introgression, wild chickpea, cultivars, stress tolerance indices

The 200 lines were seeded at two seeding dates (normal and late seeding) at three locations to expose the lines to high temp during flowering

Location	Year	Max T (°C) at flowering	
		Seeding date 1 (normal)	Seeding date 2 (late)
Moose Jaw, SK	2019	25.4	35.4
Lucky Lake, SK	2019	26.9	34.2
Lucky Lake, SK	2020	25.8	34.7
Yuma, AZ, USA	2020	29.4	38.9

RCBD with 3 reps was used at each location and seeding date

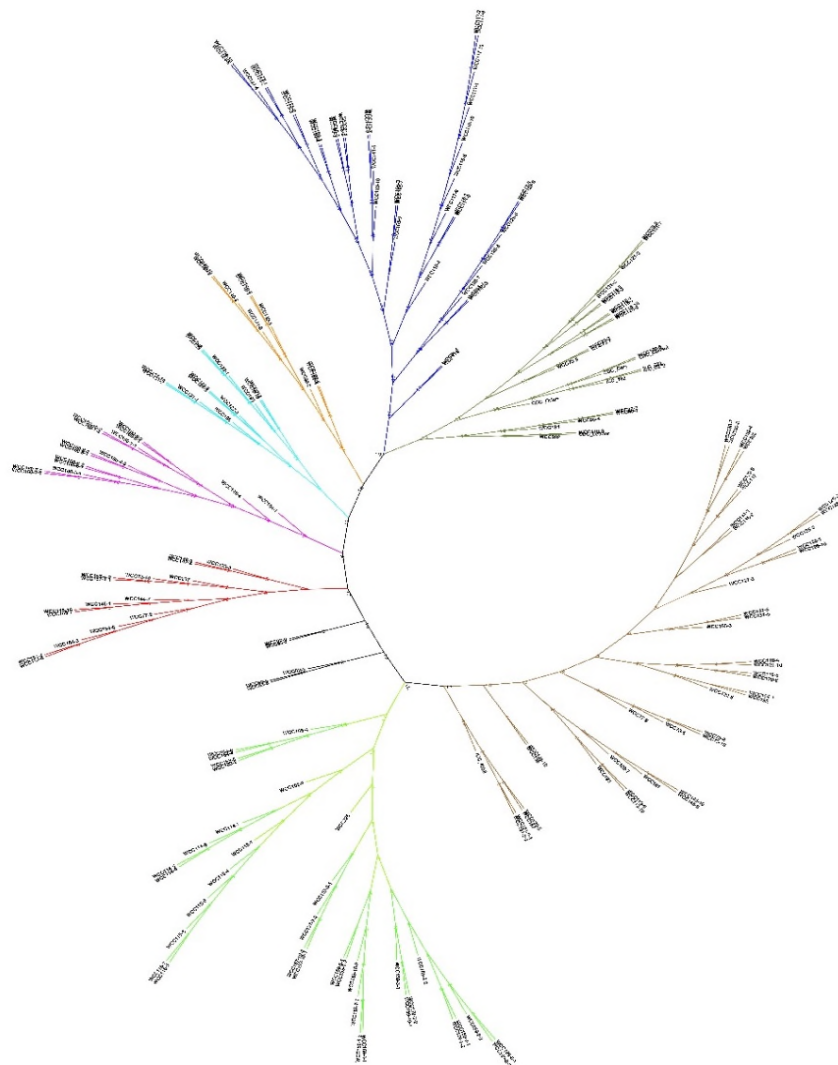
TOL (tolerance index) = $Y_p - Y_s$ (Rosielle and Hamblin, 1981)

MP (mean productivity) = $(Y_p + Y_s)/2$ (Rosielle and Hamblin, 1981)

ATI (abiotic tolerance index) = $[(Y_p - Y_s)/(\bar{Y}_p - \bar{Y}_s)] \times 100$ (Moosavi et al., 2008)

SSPI (stress susceptibility percentage index) = $[(Y_p - Y_s)/(2\bar{Y}_p)] \times 100$ (Moosavi et al., 2008)

K₁STI (modified stress tolerance index) = $(Y_p^2 / \bar{Y}_p^2) \times [(Y_p + Y_s) / \bar{Y}_p^2]$ (Farshadfar and Sutka, 2003)



Neighbor-joining tree based on 35,432 SNPs showing the genetic relatedness among the 200 chickpea interspecific inbred lines.

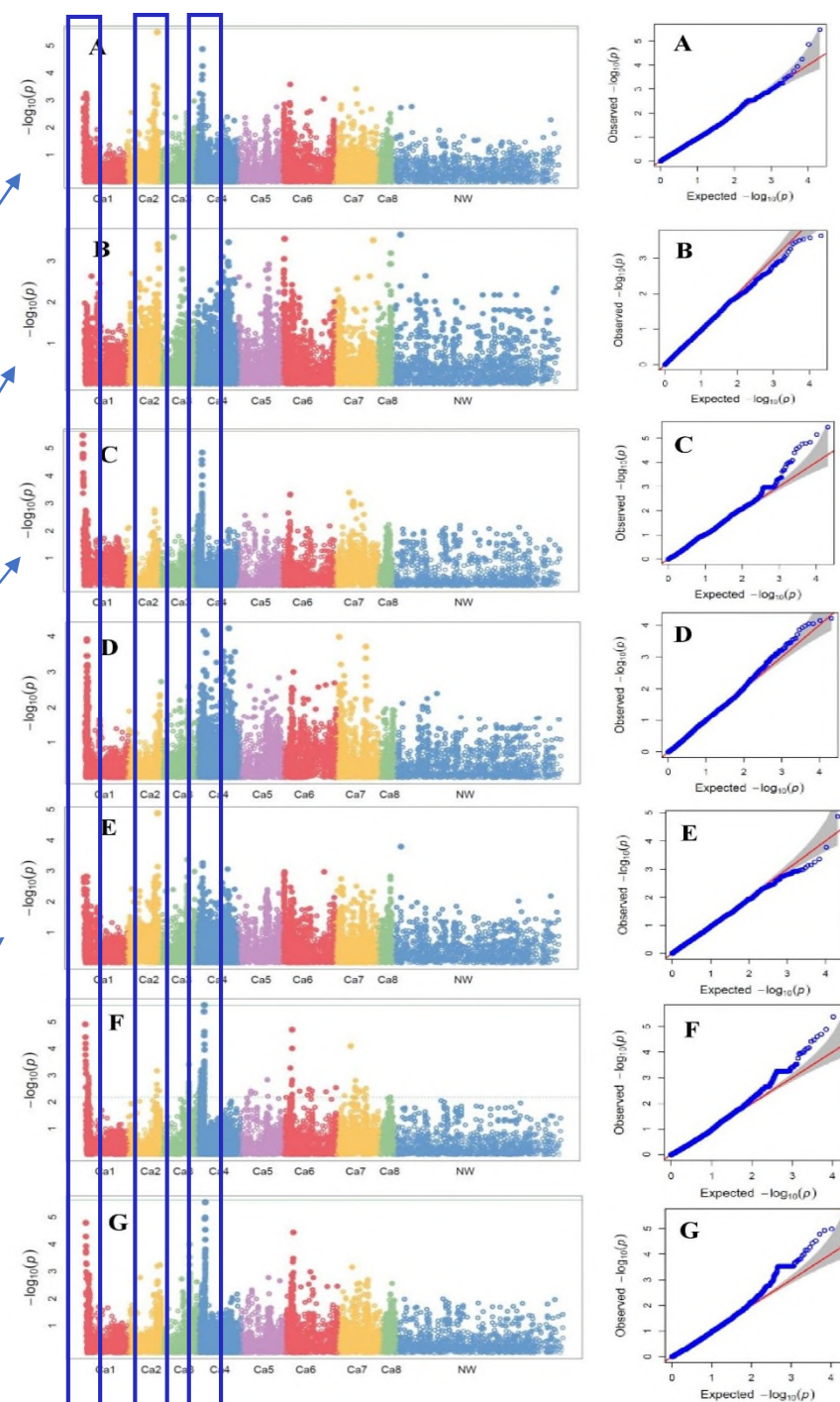


- Interspecific population chickpea nursery at Yuma, AZ.
March 12, 2020

Location	Yield (g/m ²)			
	SD1		SD2	
	Mean	Range	Mean	Range
Lucky Lake, 2019	228	28-443	193	22-395
Moose Jaw, 2019	306	90-486	210	43-385
Yuma 2019-2020	259	5-1638	89	3-282
Lucky Lake 2020	129	6-466	146	9-467

SNP markers associated with different stress indices

Stress Indices	SNP marker	P-value	MAF
Ys (Seed yield under stress conditions)	NW_9270594	2.31E-04	0.31
	Ca3_15304269	2.61E-04	0.09
	Ca6_3396299	2.89E-04	0.31
	Ca7_43614232	3.14E-04	0.41
	Ca4_37419513	3.47E-05	0.40
	Ca2_34600347	3.92E-04	0.07
Yp (Seed yield under non-stress conditions)	Ca2_34600347	3.25E-06	0.07
	Ca4_8694304	1.35E-05	0.05
	Ca4_8737135	5.55E-05	0.14
ATI (Abiotic tolerance index)	Ca1_47259	3.42E-06	0.46
	Ca1_56428	6.91E-06	0.46
K ₁ STI (Modified stress tolerance index)	Ca4_36637574	6.05E-05	0.10
	Ca4_8646741	7.12E-05	0.16
	Ca4_11276937	9.00E-05	0.07
	Ca4_11277513	9.00E-05	0.07
MP (Mean productivity)	Ca2_34600347	1.34E-05	0.07
SSPI (Stress susceptibility percentage index)	Ca4_8694304	2.37E-06	0.05
	Ca4_8313845	4.17E-06	0.06
TOL (Tolerance index)	Ca4_8694304	2.87E-06	0.05
	Ca4_8670257	1.04E-05	0.06
	Ca4_8313845	1.19E-05	0.06
	Ca1_47259	1.66E-05	0.46

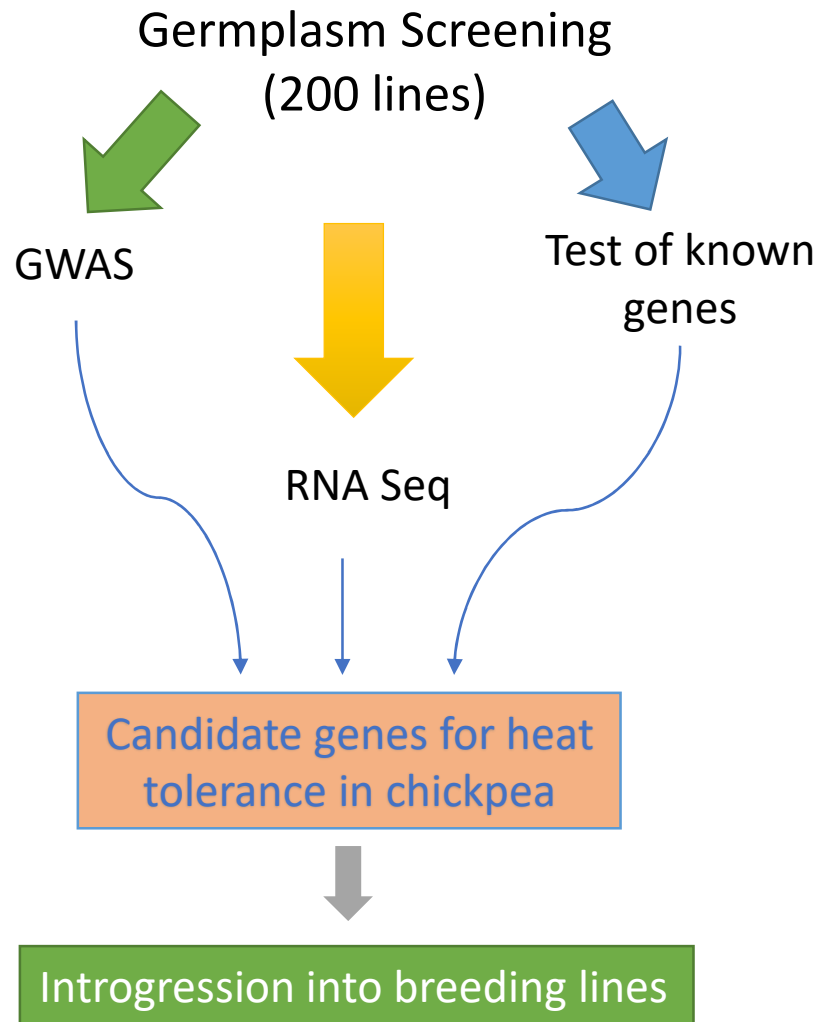




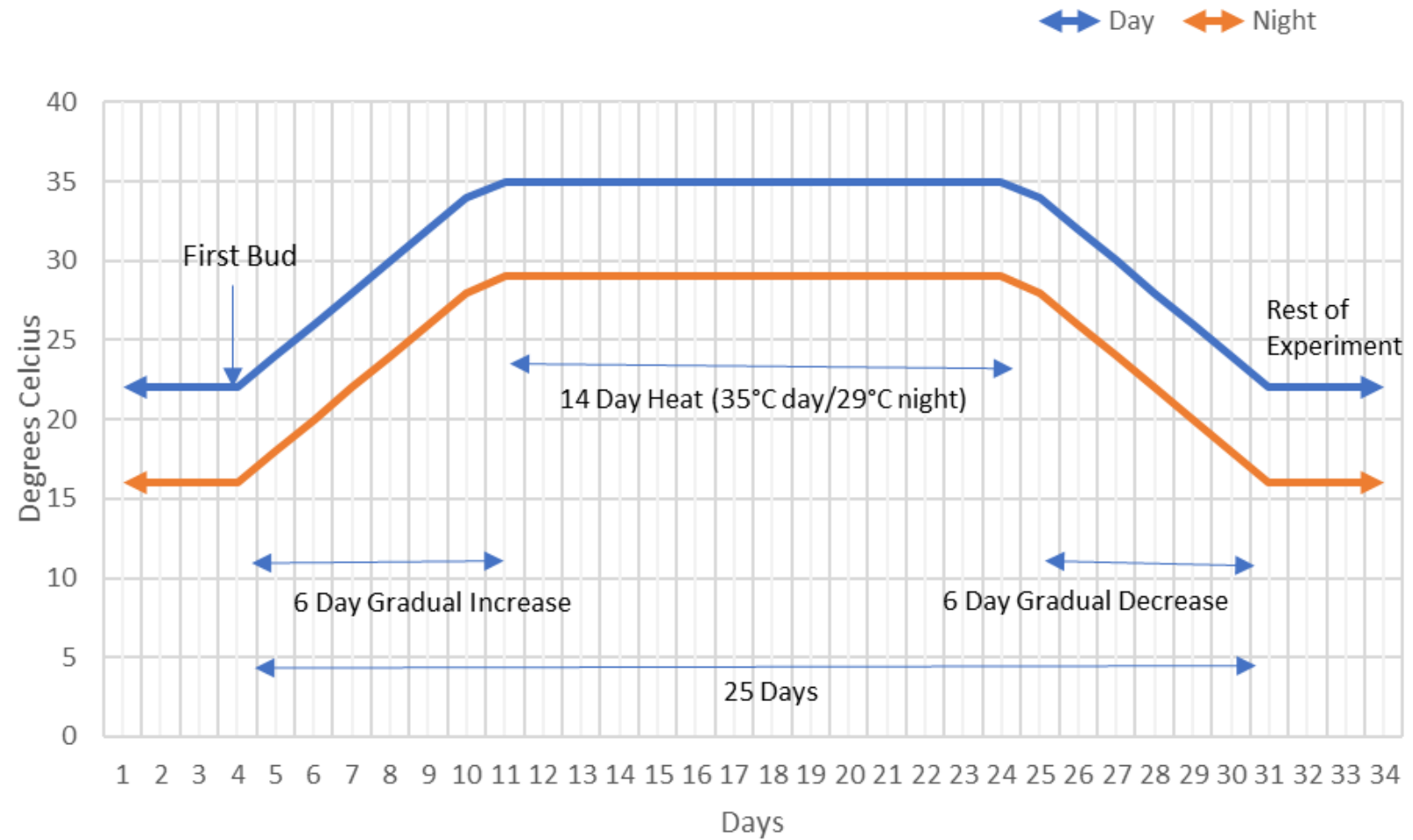
- **Genetic analysis of heat stress tolerance in chickpea**

Sophie Duchesne
(PhD student)





Evaluation for tolerance to heat stress in chickpeas



Seed Quality





Tamanna Jahan
(PhD)



frontiers

Frontiers in Plant Science

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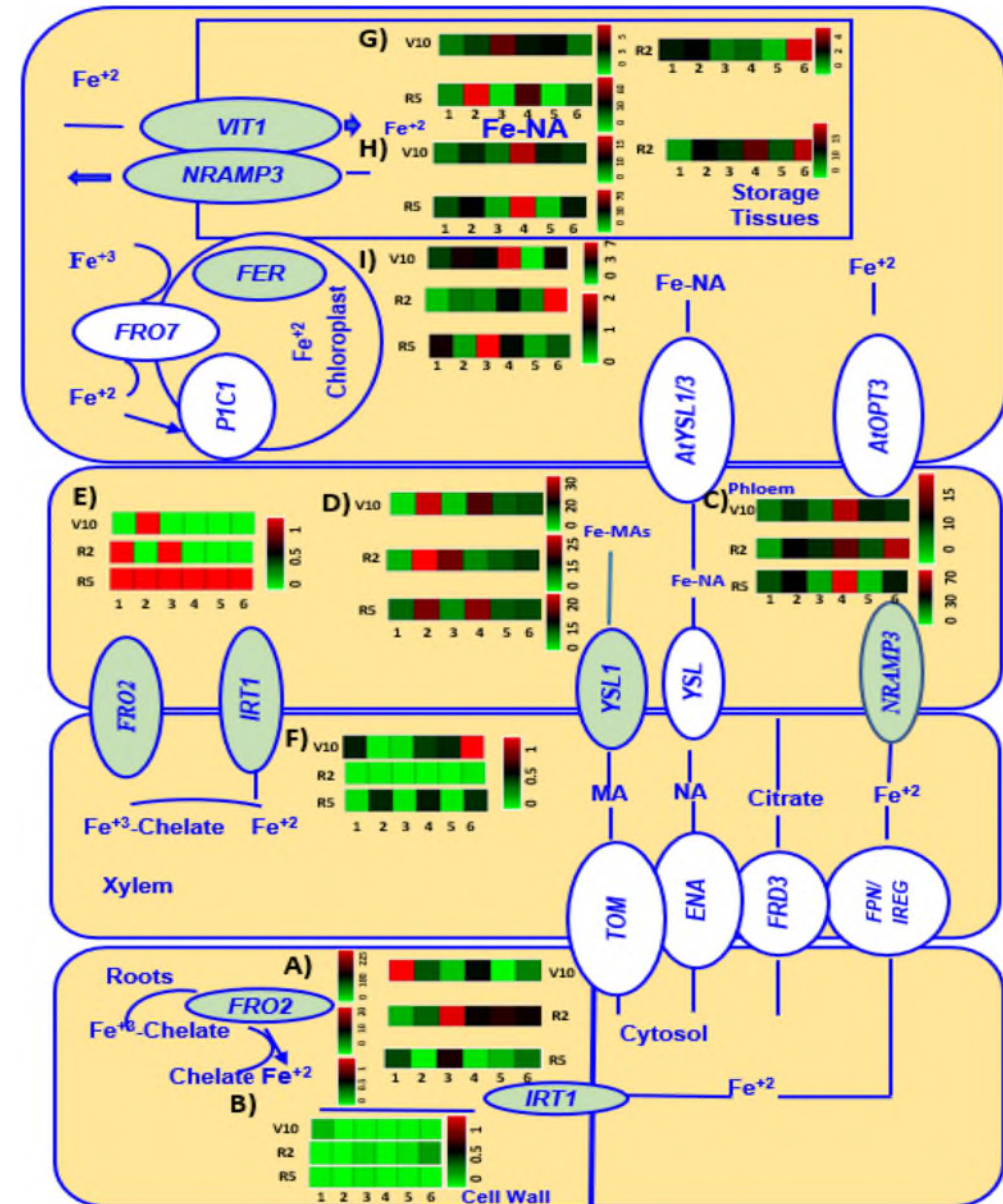
Iron accumulation and partitioning in hydroponically grown wild and cultivated chickpea (*Cicer arietinum* L)

Tamanna A. Jahan¹, Shweta Kalve¹, Zachery Belak²,
Christopher Eskiw² and Bunyamin Tar'an^{1*}

¹Department of Plant Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada, ²Department of Food and Bioproduct Sciences, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada

A heatmap analysis showing the gene expression patterns of Fe metabolism related genes FRO2 (A, E), IRT1 (B, F), NRAMP3 (C, H), YSL1 (D), VIT1 (G), and FER3 (I) in roots and leaves of six different genotypes (1 = CDC Verano, 2 = Cerri 075, 3 = FLIP97-677C, 4 = Sarik 067, 5 = Kalka 064, and 6 = CDC 551-1). The data at V10, R2 and R5 growth stages taken only from Fe added (Fe+) conditions. Green and red color represents down-regulation, and up-regulation in the color scale, respectively.

Jahan et al. 2023



Genomics & New Approaches

Genomic Selection

Of Seed Protein and Oil Content

Training GS Models

Parental lines and
germplasm
(*Founding alleles;*
 $n = 184$)

Marker Density:

MAF 5% (14,971)
MAF 10% (11,397)
LD 0.25 (1,699)
LD 0.50 (2,650)
LD 0.75 (3,785)
All markers (28,177)

Model:

RR-BLUP
G-BLUP
BayesB
BayesC*Pi*

Calculate GEBV



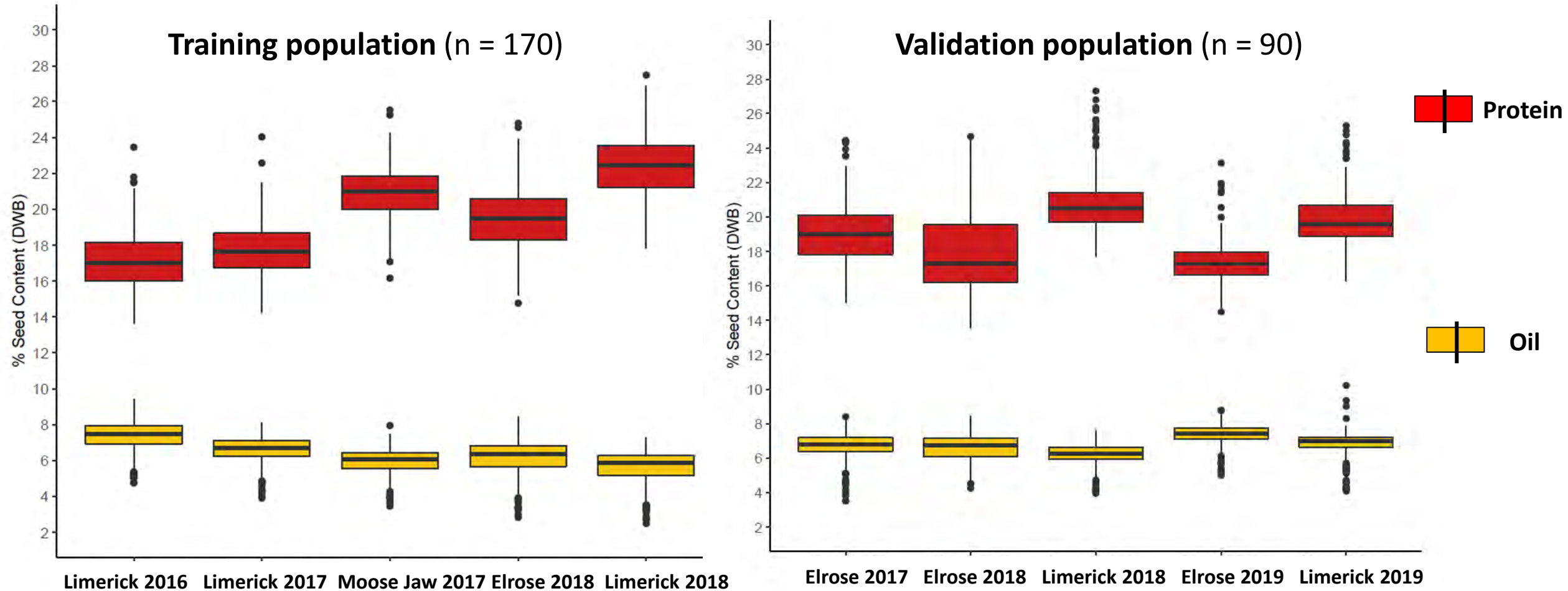
Testing GS Models

Validation population
(all elite lines from the
CDC breeding program;
 $n = 100$)

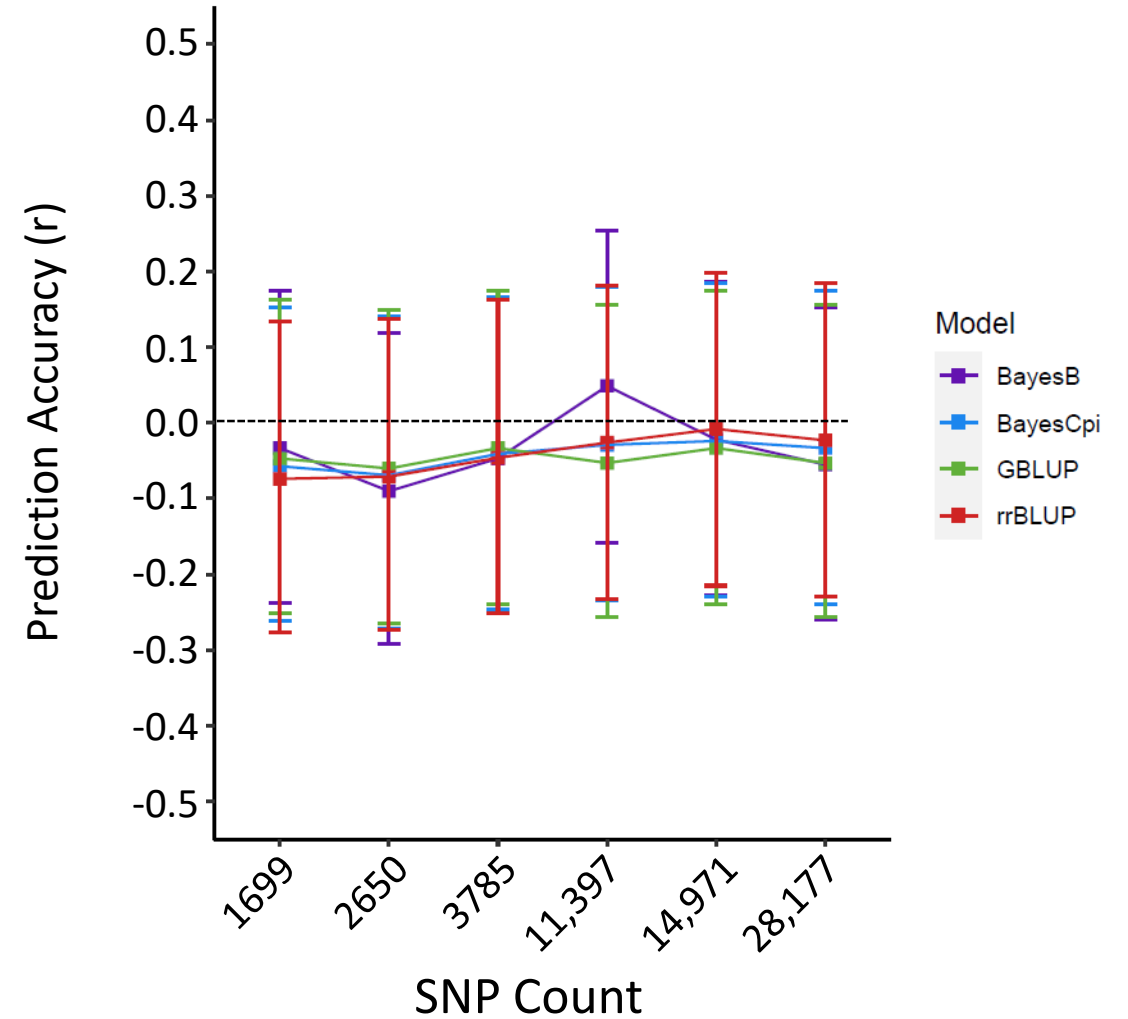
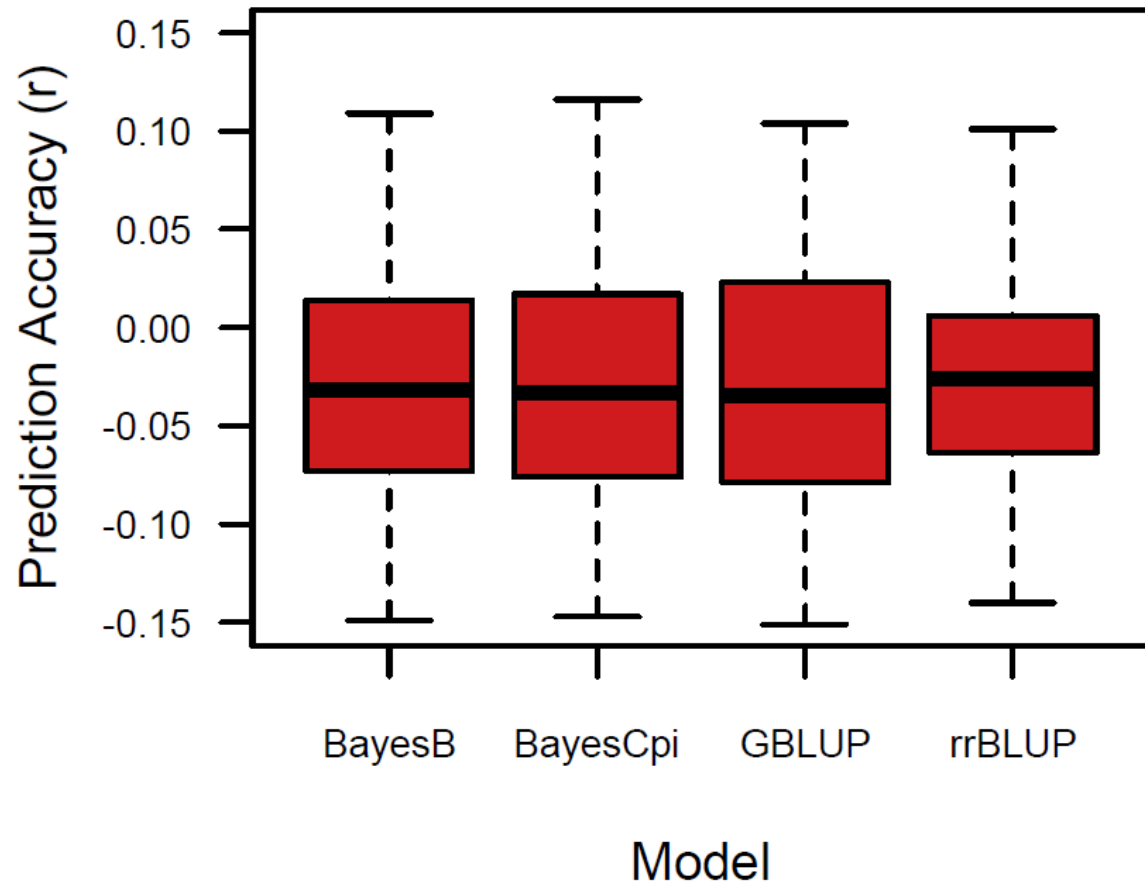


Alanna Orsak
MSc

Protein and oil contents of TP and VP at each environment (site-year)

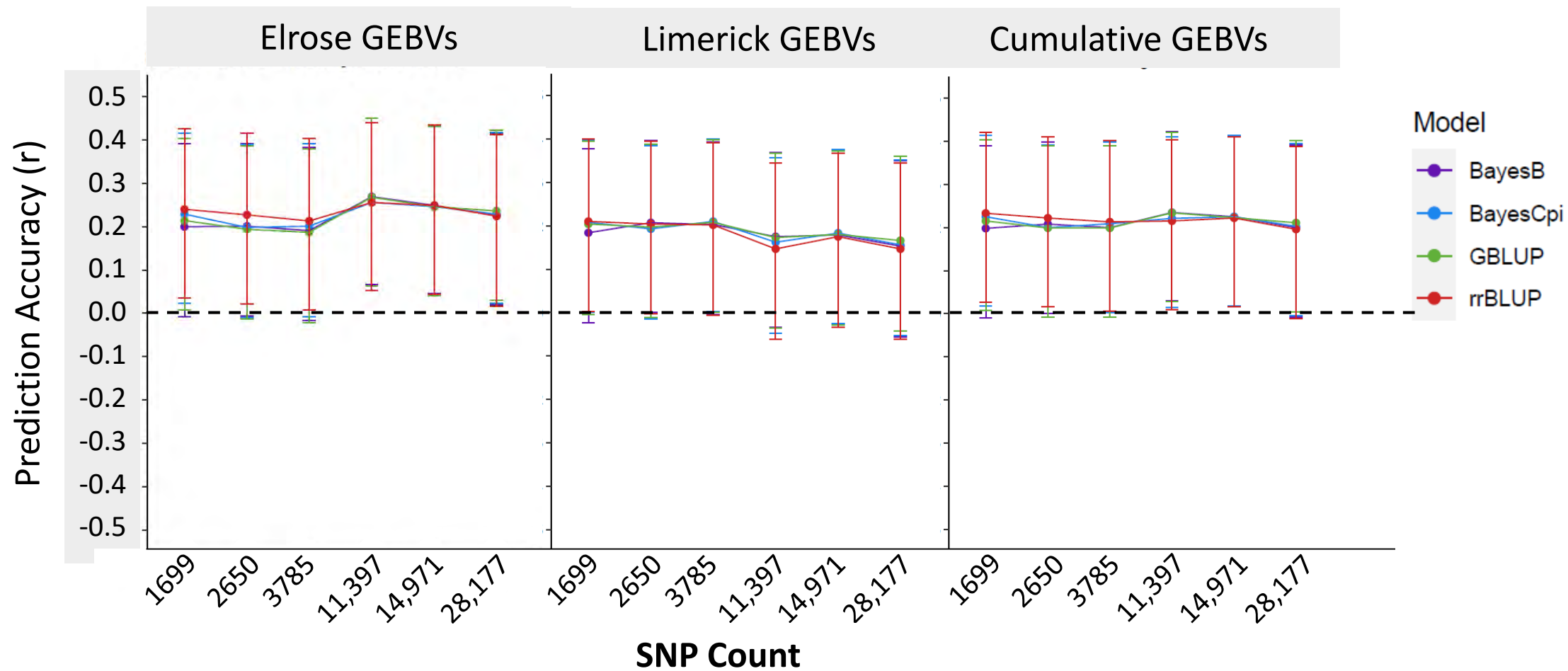


GS for protein → Zero prediction accuracy regardless of GS models and marker density



GS for Seed Oil

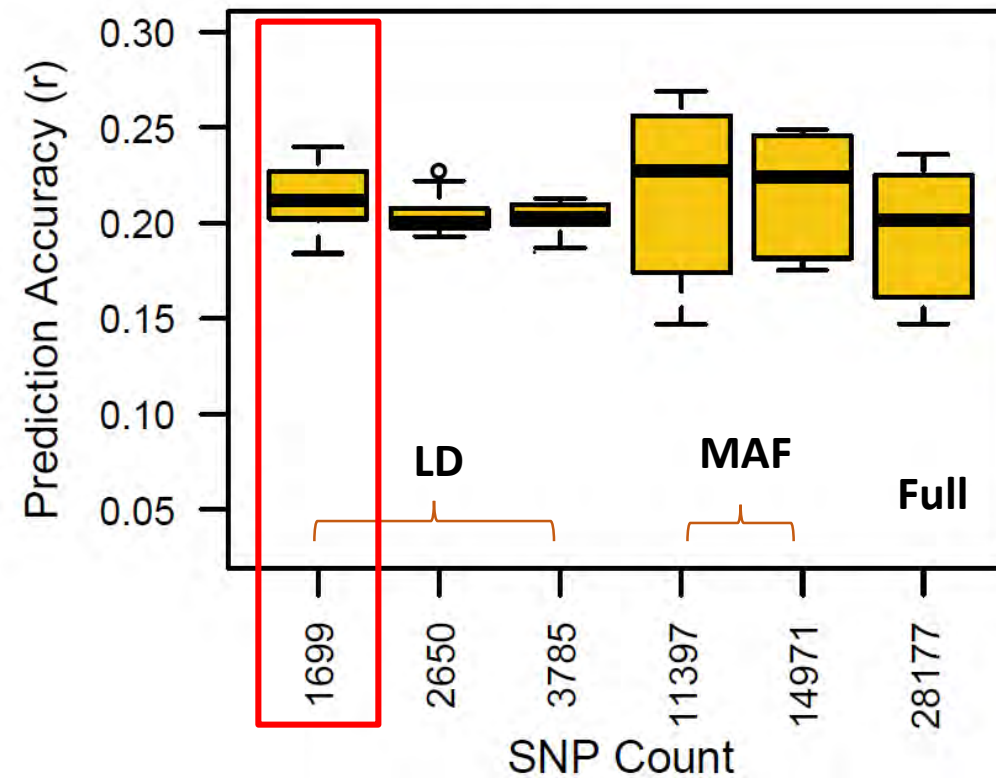
Oil with Models Trained Using Location BLUPs



Oil Prediction Models Accuracies

Limerick BLUPs predicting cumulative GEBVs at various SNP densities

Trained by Limerick BLUPs



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A chickpea genetic variation map based on the sequencing of 3,366 genomes

[Rajeev K. Varshney](#) , [Manish Roorkiwal](#), [Shuai Sun](#), [Prasad Bajaj](#), [Annapurna Chitikineni](#), [Mahendar Thudi](#), [Narendra P. Singh](#), [Xiao Du](#), [Hari D. Upadhyaya](#), [Aamir W. Khan](#), [Yue Wang](#), [Vanika Garg](#), [Guangyi Fan](#), [Wallace A. Cowling](#), [José Crossa](#), [Laurent Gentzbittel](#), [Kai Peter Voss-Fels](#), [Vinod Kumar Valluri](#), [Pallavi Sinha](#), [Vikas K. Singh](#), [Cécile Ben](#), [Abhishek Rathore](#), [Ramu Punna](#), [Muneendra K. Singh](#), [Bunyamin Tar'an](#), [Chellapilla Bharadwaj](#), [Mohammad Yasin](#), [Motisagar S. Pithia](#), [Servejeet Singh](#), [Khela Ram Soren](#), [Himabindu Kudapa](#), [Diego Jarquín](#), [Philippe Cubry](#), [Lee T. Hickey](#), [Girish Prasad Dixit](#), [Anne-Céline Thuillet](#), [Aladdin Hamwieh](#), [Shiv Kumar](#), [Amit A. Deokar](#), [Sushil K. Chaturvedi](#), [Aleena Francis](#), [Réka Howard](#), [Debasis Chattopadhyay](#), [David Edwards](#), [Eric Lyons](#), [Yves Vigouroux](#), [Ben J. Hayes](#), [Eric von Wettberg](#), [Swapn K. Datta](#), [Huanming Yang](#), [Henry T. Nguyen](#), [Jian Wang](#), [Kadambot H. M. Siddique](#), [Trilochan Mohapatra](#), [Jeffrey L. Bennetzen](#), [Xun Xu](#) & [Xin Liu](#)  [-Show fewer authors](#)

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With Rajeev Varshney
Senior author

Highlights of the paper:

Developed Haplotype map in chickpea based on whole genome sequencing of 3,366 chickpea germplasm accessions including 195 accessions from seven wild species of the primary, secondary and tertiary gene pools

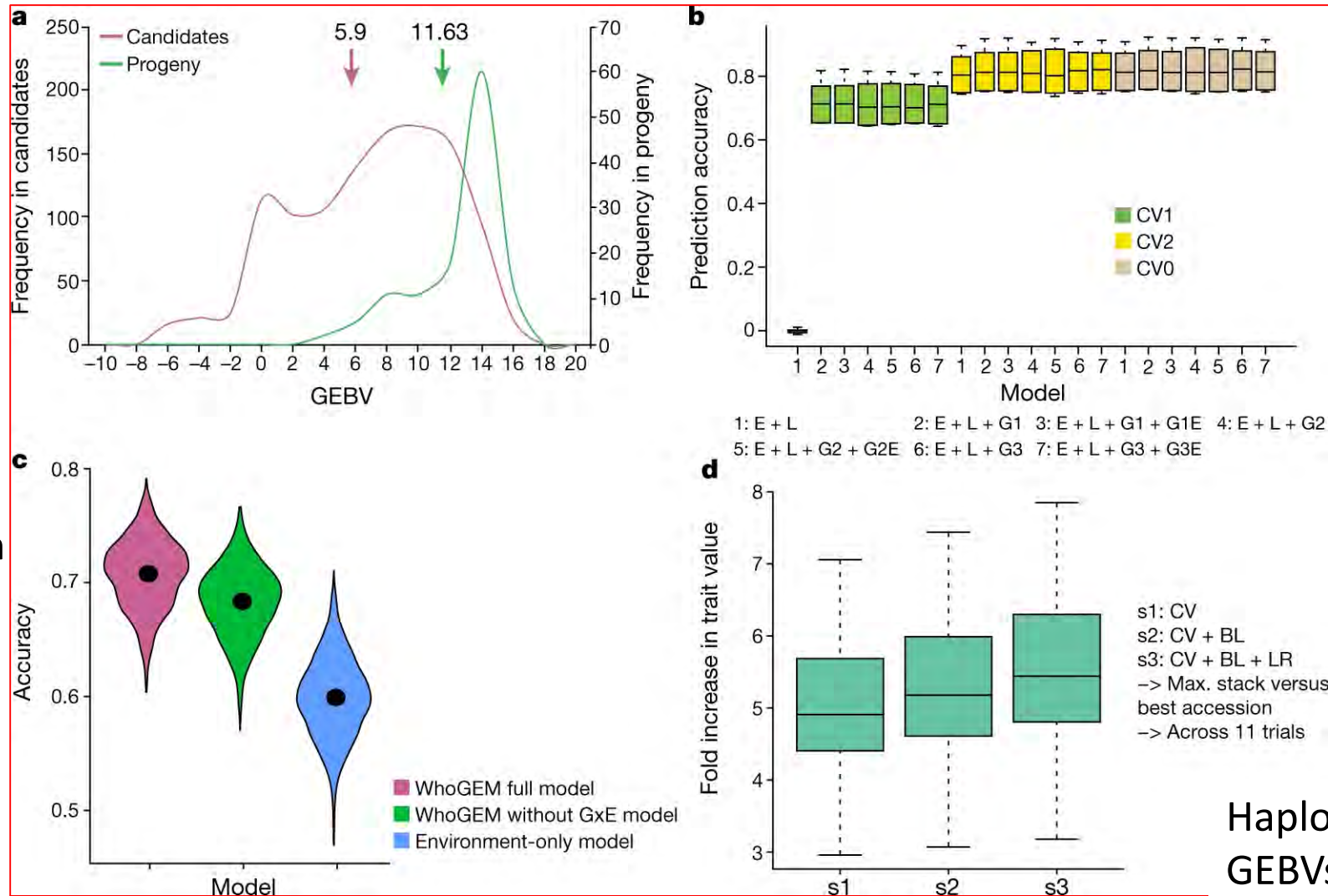
Developed Cicer pangenome based on the largest number of genotypes

Developed and optimized genomic breeding approaches, haplotype-based breeding, genomic prediction, and optimal contributions selection for developing tailor-made high yielding and climate resilient chickpea varieties.

Different strategies of genomic breeding for improving 100-seed weight in chickpeas.

Mean GEBV
→ 23% increase
in one generation
for seed size.

A general linear
model using the
WhoGEM prediction
machine → highest
prediction
accuracies for the
full model
($n = 1,500$; 300
replicates of a
fivefold cross-
validation).



GEBV based on
Bayesian
generalized linear
regression (BGLR)
→ highest mean
prediction
accuracy ($n = 2,980$
cultivated
accessions).

Haplotype-based local
GEBVs that are suggested
to provide a fivefold gain
over GEBV



+

Flax Breeding and Genetics

•

○



Breeding objectives and goals:



- Yield
- Early maturity
- Disease resistances (maintenance and improvement)
- Straw management
- Lodging resistance
- Acceptable seed quality
- Abiotic stress tolerance



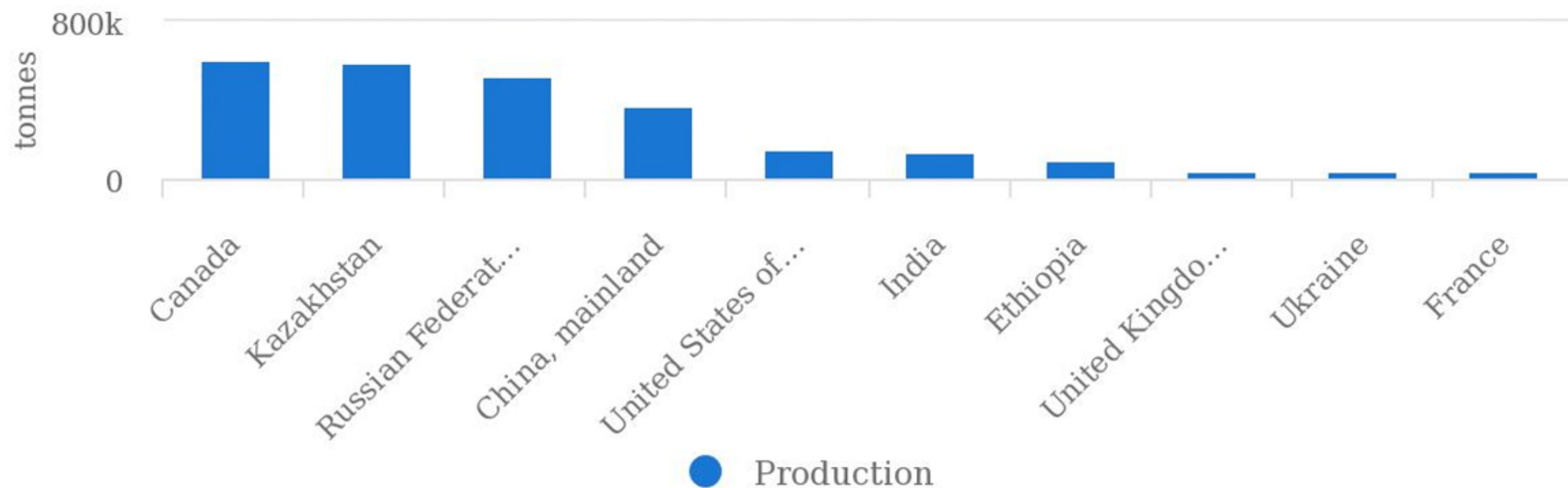
- Total oil Content ~50%,
- ALA: 60 - 70% of oil,
- High seed protein ~30% DSW (>60% of Meal)
- Plant stature



- Reducing anti-nutritional compounds (e.g. low cyanogenic glycosides)
- Low cadmium

Production of Linseed: top 10 producers

Average 2011 - 2020



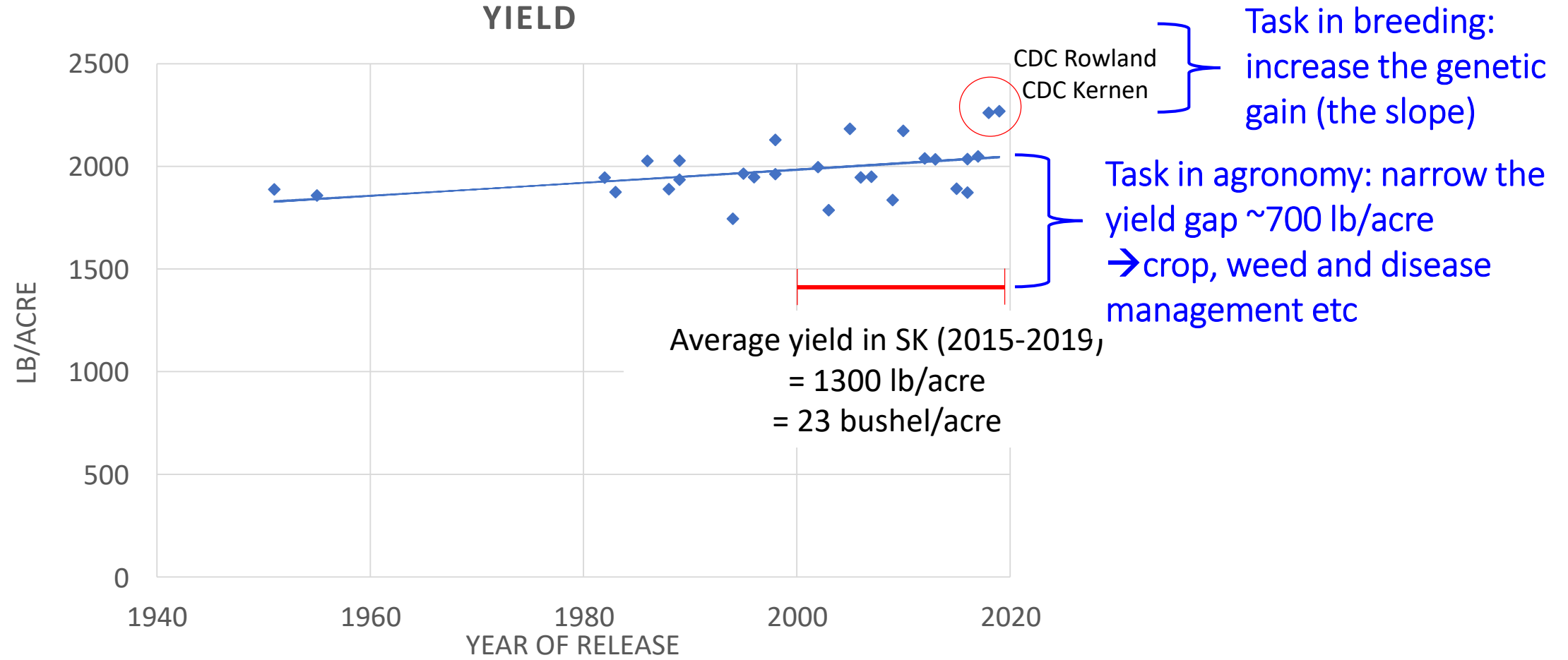
Source: FAOSTAT (Jan 07, 2022)

Country	Average yield 2015-2020	
	(kg/ha)	(lb/acre)
Canada	1474	1315
China	1311	1170
United States of America	1289	1150
Russian Federation	868	774
Kazakhstan	823	734
India	540	482

→ Ideally: 1800 lb/acre

Source: FAOSTAT Jan 07, 2022

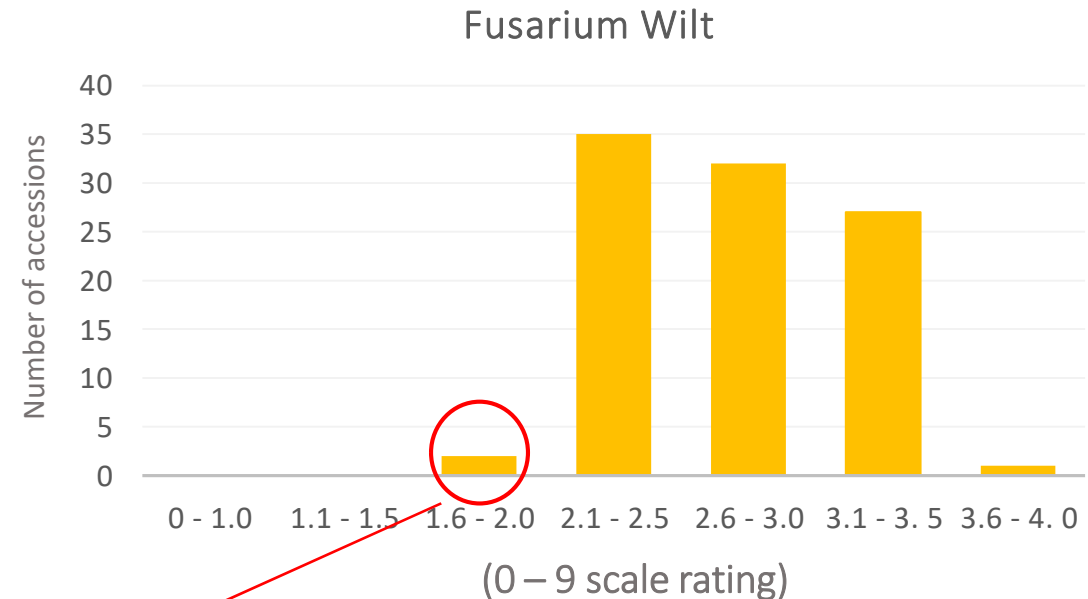
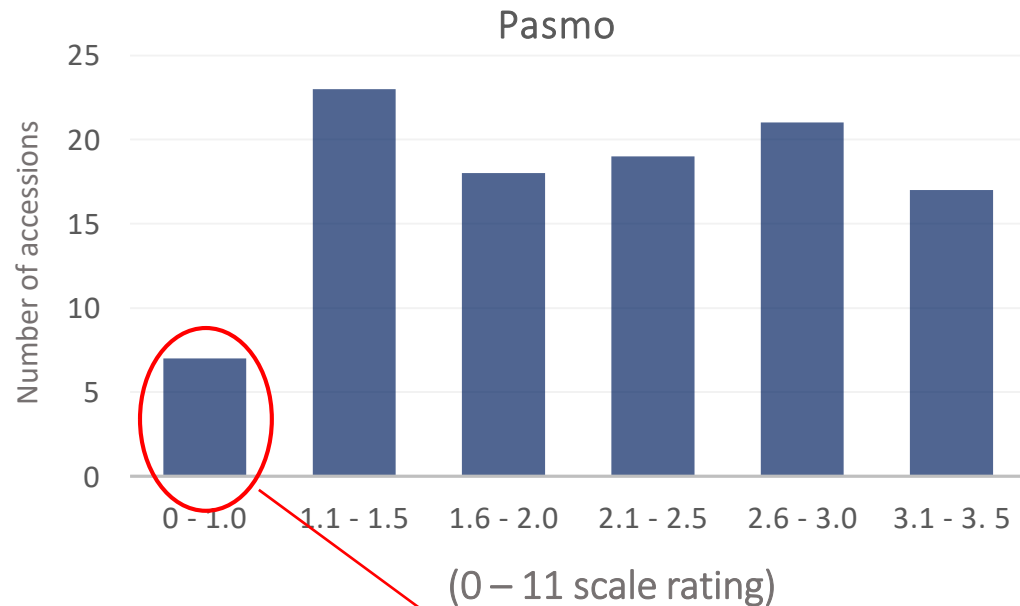
Average yield of the old and newer flax cultivars vs Average yield at farms in SK



Improvement of resistance to pasmo and fusarium wilt in flax

(Q 2)

Frequency distribution of top 100 flax accessions from PGRC for their reaction to pasmo and fusarium wilt (average of 2014-2019 trials)



2020 crossing block

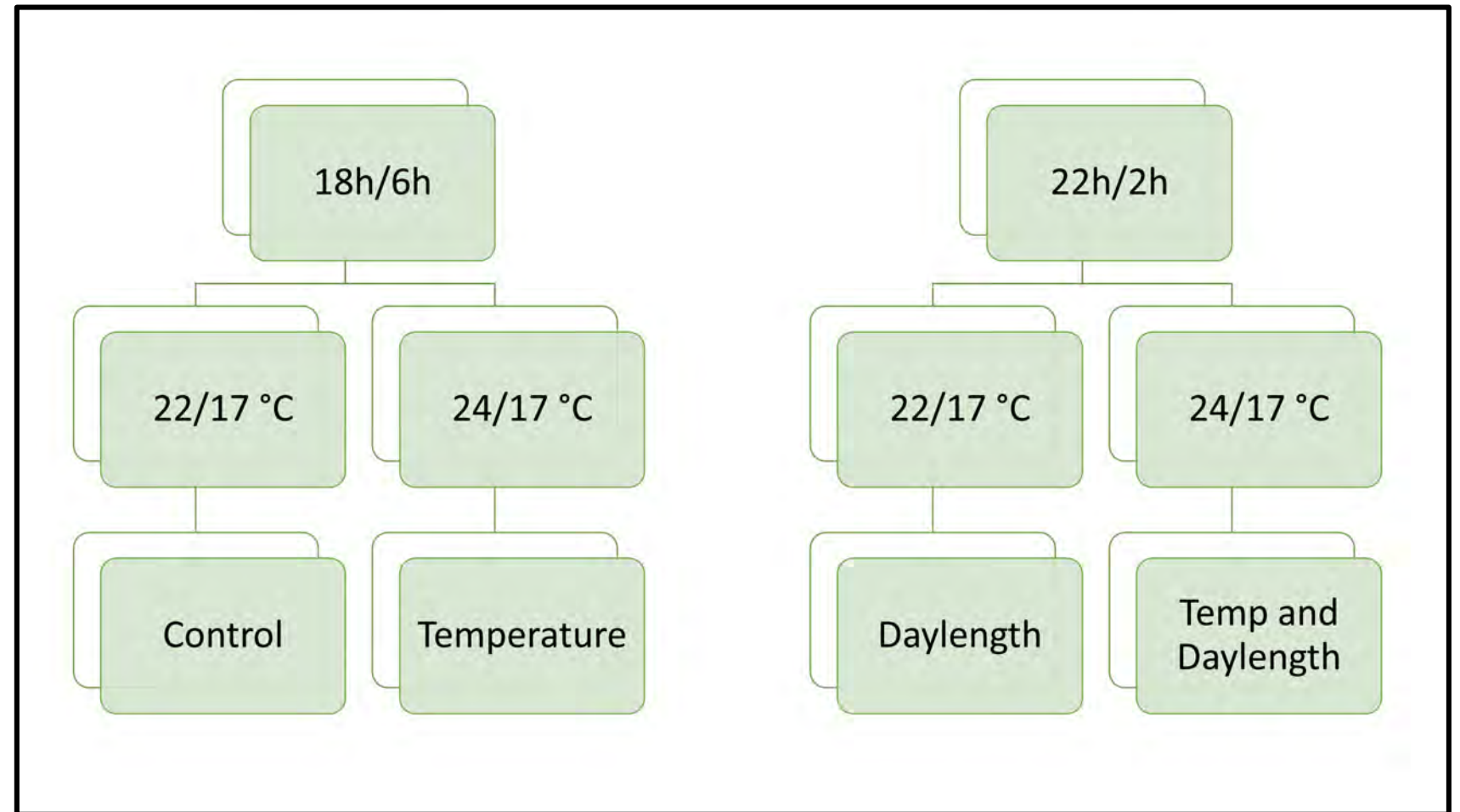
Pasmo: 65 populations
Fusarium wilt: 68 populations
2023: F4/F5 populations (KCRF)

Pasmo and fusarium wilt screening was done by the CDC Flax Pathology program (Dr. Randy Kutcher)

ADF#20210948 Accelerated Breeding Strategy for Flax Improvement (Matching funds: WGRF, SaskFlax, MCA)



Dr. Megan House
Research Officer



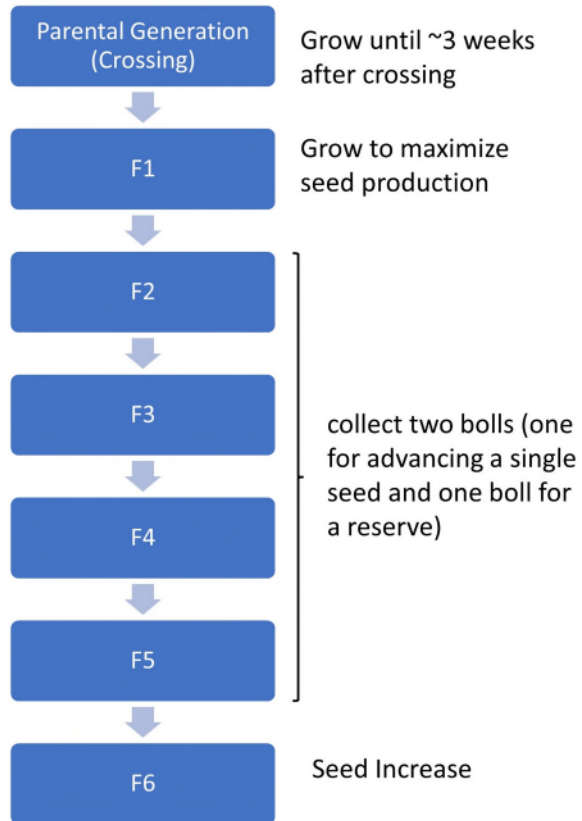
System optimization

ADF#20210948

Accelerated Breeding Strategy for Flax Improvement

RIL Development

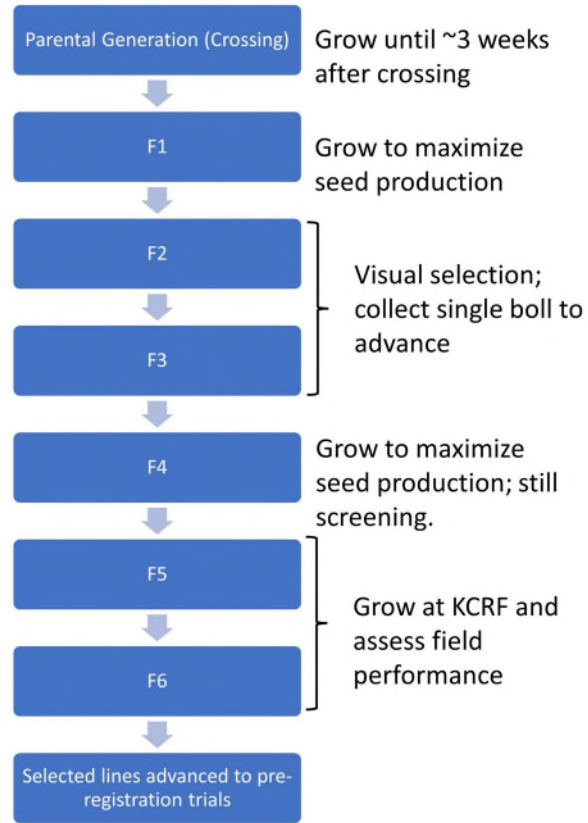
(Pasco and Fusarium wilt)



Quick population development

Short & Large Seed Type x CDC Kernan (or FP2591)

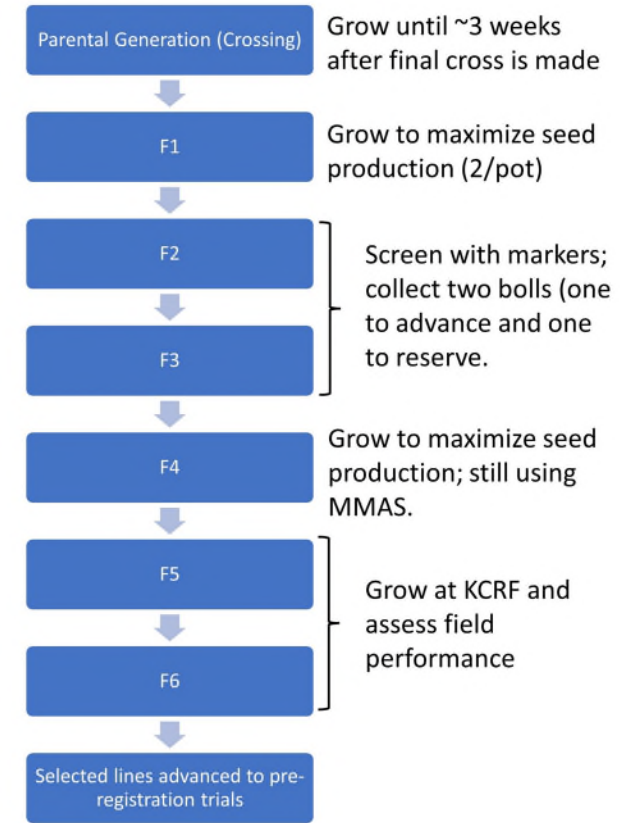
(short with large seeds) x (elite variety)



Integration with phenotypic selection

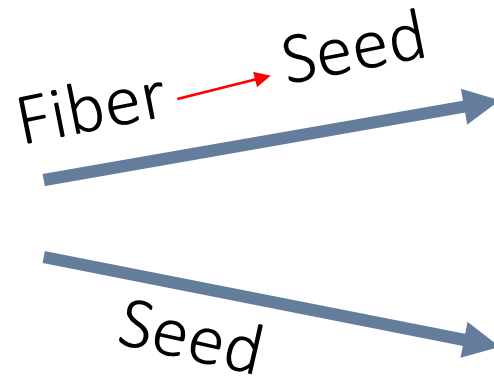
Adelie x CDC Dorado

(brown seed coat/rust susceptible, powdery mildew resistant) x (yellow seed coat, rust resistant, and PM susceptible)



Integration with MMAS

Mining wild relatives



The Use of Wild *Linum* Species for Genetic Improvement of Resistance to PasmO in the Cultivated Flax

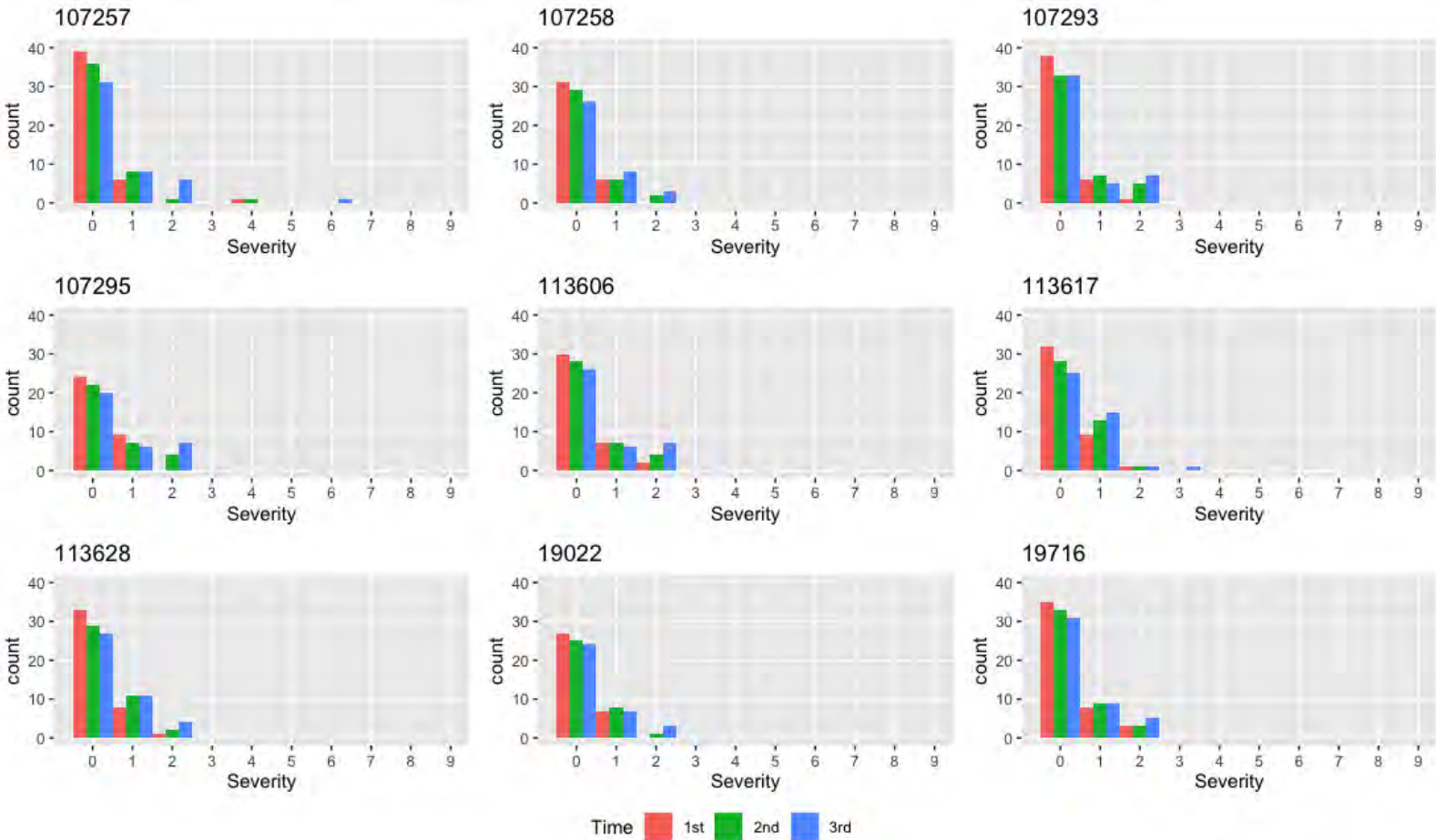
- Expand germplasm base/increase diversity
- Source for disease resistance (pasmO)
- Source for abiotic stress tolerance
- Source for yield component improvement



Xinjie Yu
(PhD student)



Page 10 of 10



Boll dehiscence

- L. bienne*



<i>L. bienne</i>		
Accessions	Origin	Dehiscent Level
PGRC CN 107293	unknown	6
PGRC CN 113617	Turkey	7
PGRC CN 19022	Germany	7
PGRC CN 107295	Greece	8
PGRC CN 107258	unknown	8
PGRC CN 19716	Greece	8
PGRC CN 113628	Turkey	8
PGRC CN 113606	Turkey	8
PGRC CN 107257	unknown	9

- CDC Bethune



Rating scale:

Indehiscent

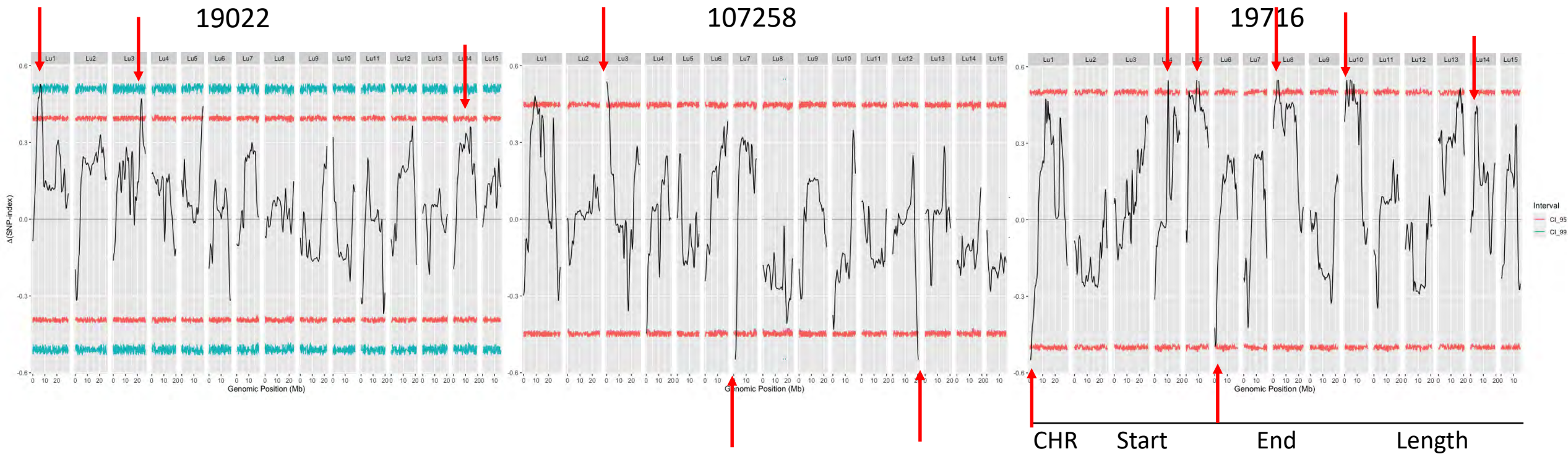
Dehiscent



1

10

QTLs associated with boll dehiscence identified using bulked segregant sequencing



CHR	Start	End	Length
Lu1	4093034	7472902	3379868
Lu3	22519712	23959006	1439294
Lu14	13836257	14021155	184898

CHR	Start	End	Length
Lu3	19754	872281	852527
Lu7	3623	1699825	1696202
Lu12	20132127	20887091	754964

CHR	Start	End	Length
Lu1	619	3119648	3119029
Lu4	10210827	11409814	1198987
Lu5	1763828	14573220	12809392
Lu6	19257	1653184	1633927
Lu8	89398	7074836	6985438
Lu10	29193	10981877	10952684
Lu14	3600049	5885995	2285946

Source: Xinjie Yu (2023)

Breeding objectives



Coriander *Coriandrum sativum*

- Cultivated types can be divided into two classes on basis of seed size: small and large seeded. Small seeded type are highly aromatic vs less aromatic in large seeded type

Germplasm base development

Improve resistance to blossom blight disease and yield

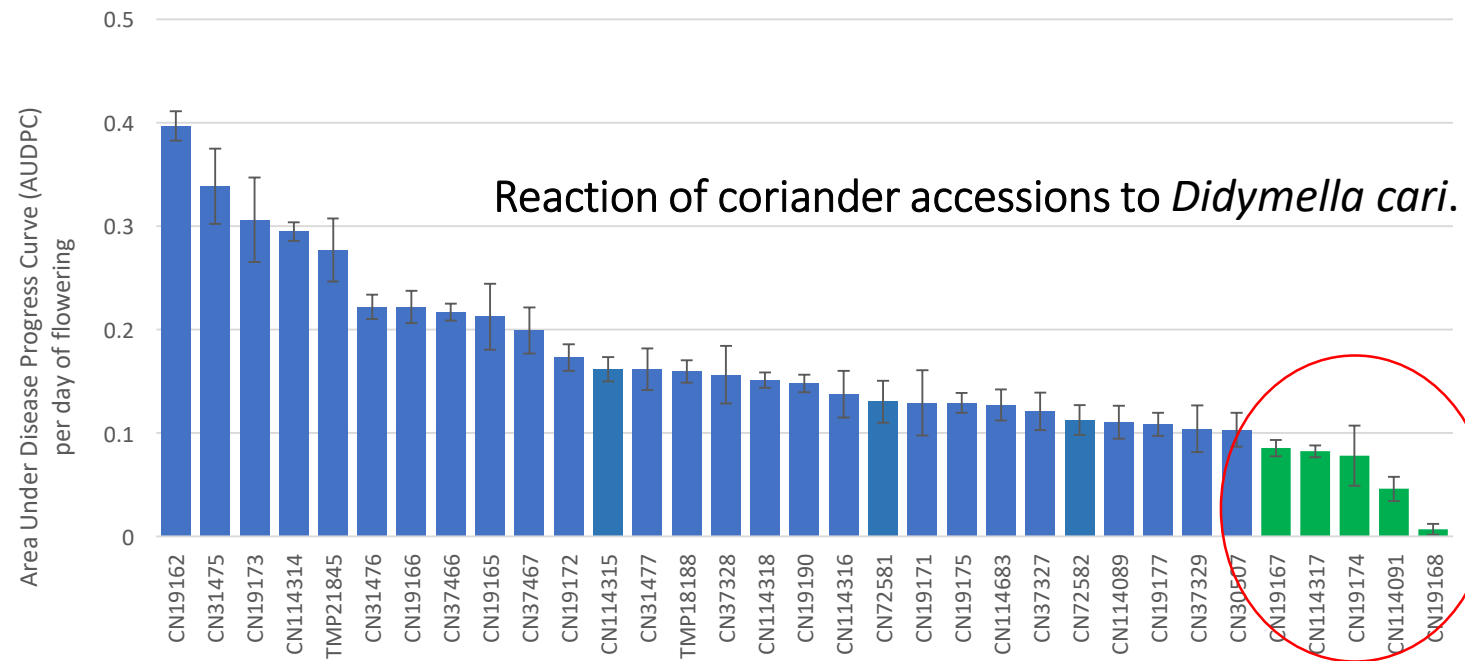
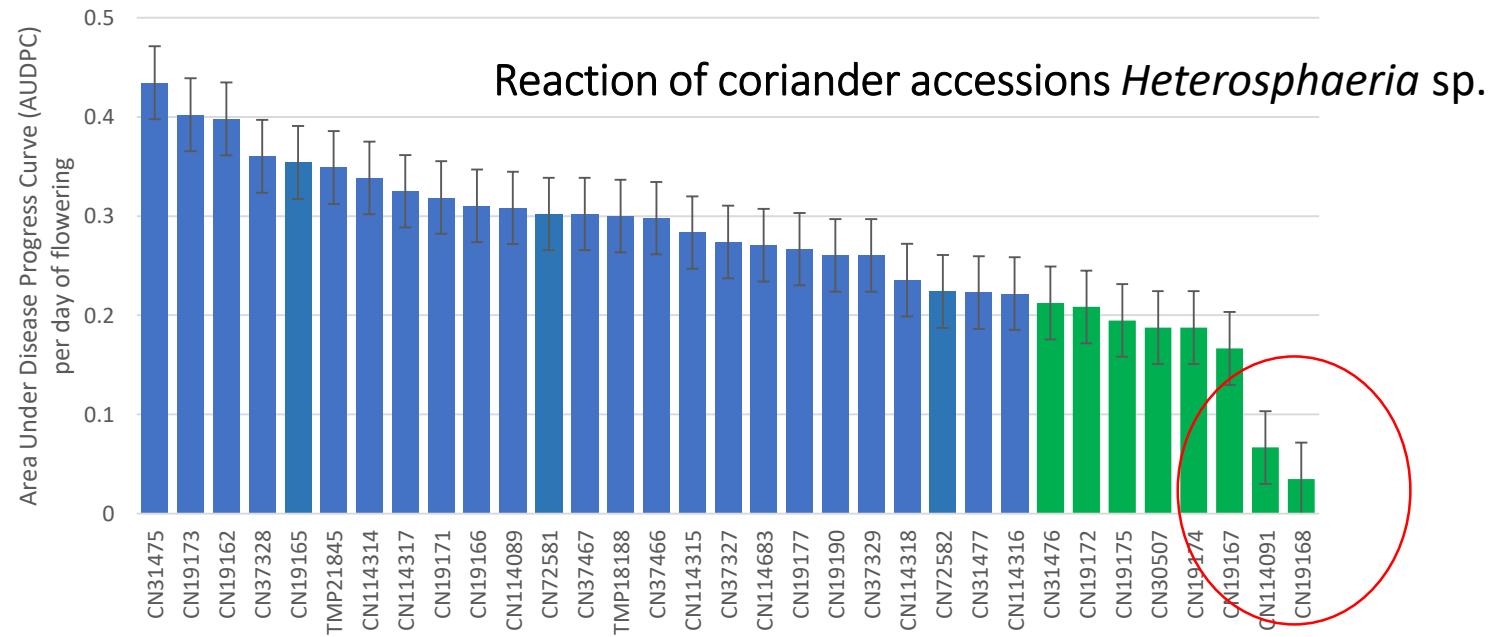
Essential oil content



Caraway *Carum carvi*

Development of germplasm base through mutation breeding

Genetic improvement of disease resistance in biennial caraway



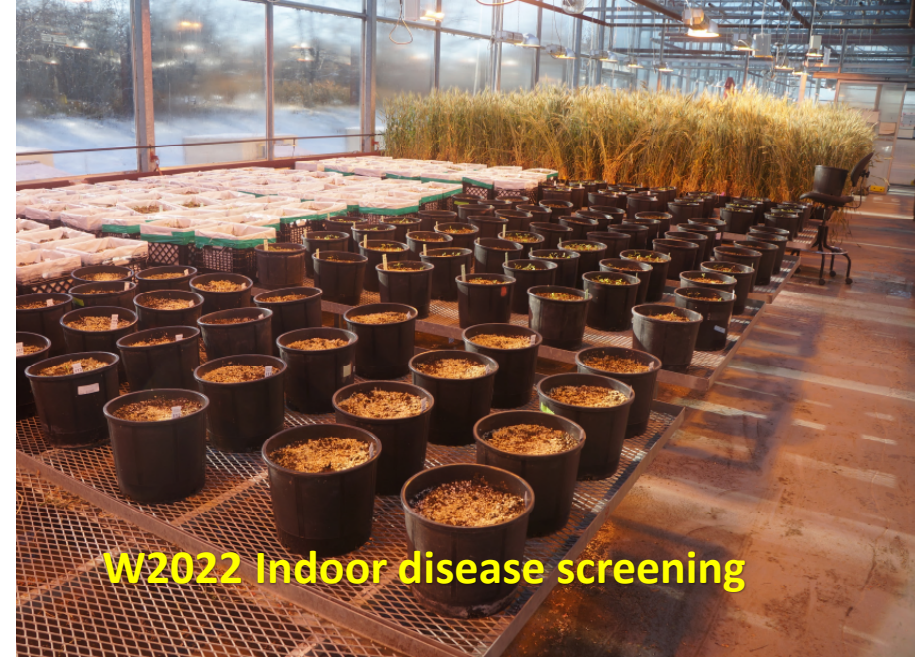
Crosses with
current
cultivars



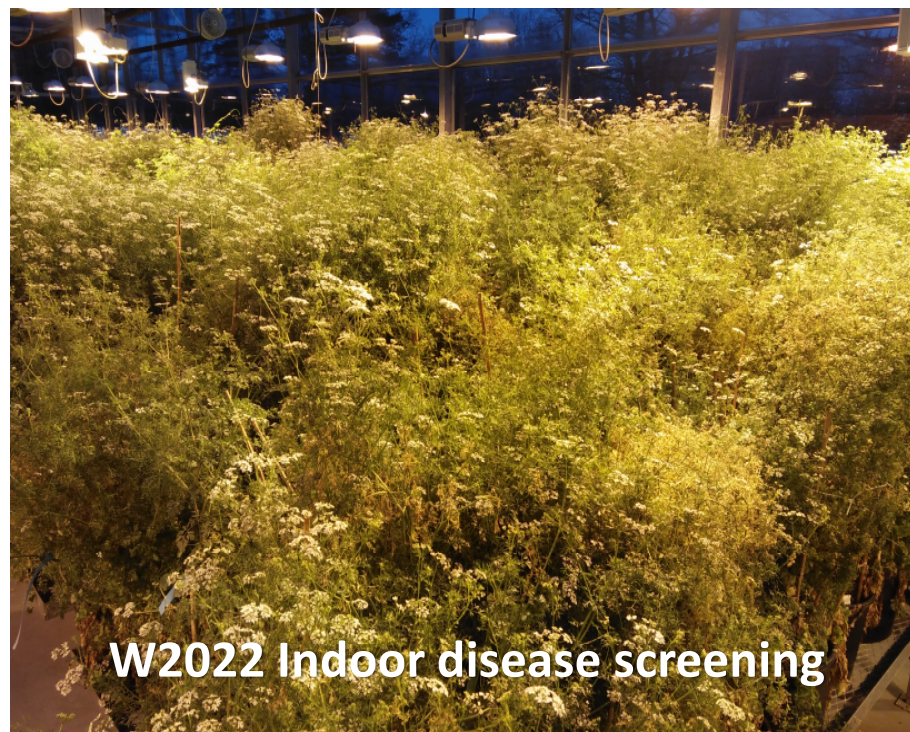
- Coriander crossing block
cross pollinated diploid species
($2n = 2x = 22$)



2021 disease screening



W2022 Indoor disease screening



W2022 Indoor disease screening

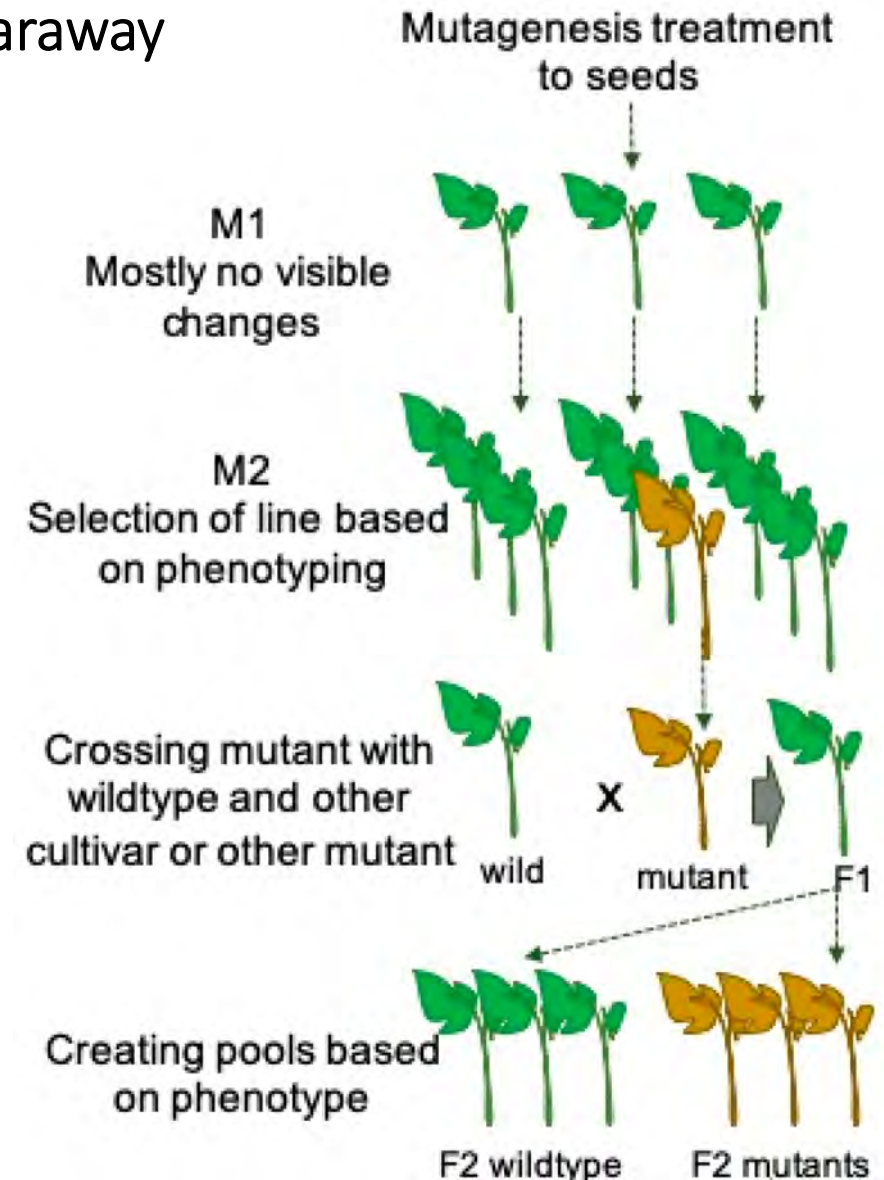


2022 Coriander disease screening : 300 Progeny Rows

Mutation breeding and pedigree selection for genetic improvement of disease resistance in biennial caraway



- Caraway (*Carum carvi* L., $2n = 2x = 20$)
- Family: Apiaceae family (syn. Umbelliferae).





Caraway M1 population
(Photo taken 3 Dec 2021)



Acknowledgements



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ALLIANCE**



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Thank You !