Kirk House Annual Meeting

University of Zambia

Genetic Dissection of Common Bacterial Blight Resistance in The Andean Gene Pool Of Common Bean

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Annual Report- Year 1

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Research Objectives

1. To determine incidence and severity of Common Bacterial Blight in major bean growing areas of Zambia.

2. To identify genomic regions and candidate genes associated with CBB resistance in the Andean Diversity Panel of common bean.

3. To map quantitative trait loci for Common Bacterial Blight resistance in an Andean population derived from a cross of Inferno (ADP 631) and Kabulangeti.

Research Objectives

4. To determine the effectiveness of CBB major-effect QTL SU91 and SAP6 against Common Bacterial Blight strains in Zambia.

5. To evaluate the yield stability and genotype × environment interaction (G×E) of Common Bacterial Blight resistant elite lines.

Diagnostic survey of Common Bacterial Blight in major bean growing areas of Zambia

Introduction

- CBB causes severe yield and quality losses.
- Genetic control is the most effective and eco-friendly way to control CBB
- However, development of resistant varieties requires an in depth understanding of the distribution and levels of damage in the production areas.
- In Zambia, the distribution and intensity of CBB in major bean growing areas is not known.
- Hence, undertook this study to give information on incidence and severity of CBB in major bean growing areas

Objective

To determine incidence and severity of Common Bacterial Blight in major bean growing areas of Zambia.

Materials and Methods

- 10 districts of Northern Zambia were targeted
- Two camps per district and three field were evaluated per camp
- Five spots per field with 20 consecutive plants were assessed for incidence and severity
- A scale of 1-5 (Benjarano 1996) was used to assess severity where;
  - 1=no symptom,
  - 2= 1-30% foliage affected
  - 3= 30-60% foliage affected
  - 4= 60-100% foliage affected
  - 5 = dead plant

Materials and Methods

- Disease incidence = number of plants infected/ total number of plants evaluated X 100

- Disease intensity Index = I x S/ M
  - Where ;
    - I = Mean disease incidence (%)
    - S= mean severity score of foliar symptoms
    - M= maximuim severity value (i.e 5)

- Farmers were also asked about source of planting seed

Analysis

Data was transformed and subjected to Analysis of Variance in GenStat discovery Software version 19.

Results and Discussion

Source of Planting seed

S/N	Source	Number of Farmers	Percentage
1	Agro-dealer/ Certified	44	73.3
2	Own seed/ Recycled	16	26.7
3	Total	60	

Results

- Highly significant differences were observes across districts
- CBB symptoms observed in all districts
- Symptoms ranged from single spots, multiple spots, edge necrosis and severe necrosis.
- Overall means
  - CBB Incidence = 90.6 (68 -100)
  - CBB intensity = 60.3 (31.8-76.8)

  - ANT incidence = 60.3 (23.5- 91.7)
  - ANT intensity = 38.8 (14.7- 68.1)

Results and Discussion

- Mean Incidence and Intensity

District	CBB DI %	ANT DI %	CBB Inc %	ANT Inc %
Luwingu	31.83a	14.7 a	68	23.5 a
Mbala	47.4ab	15.86 a	87.7	23.8 a
Kasama	49.4ab	67.15 cd	81.5	86.67 d
Lupososhi	59.17bc	68.1 d	92.8	91.67 d
Chipili	61.6bc	58.05 bcd	91.3	81.83 cd
Lunte	62.74bc	33.81 abcd	93	62.5 bcd
Kawambwa	62.91bc	49.18 abcd	91.8	71.83 bcd
Senga	74.9c	31.63 ab	100	45.83abc
Mpika	76.13c	17.35 a	100	35 ab
Mporokoso	76.8c	32.53 abc	100	80 cd
Grand mean	***60.3***	***38.8***	***90.6***	***60.3***
Pr	***0.001***	***0.01***	***0.112***	***0.001***

Conclusion

- Preliminary results showed a wide distribution of CBB (90.6 %) in the production area compared to Anthracnose (60%)

- Equally the level of damage caused by CBB (60.3 %) was more than that of anthracnose (38. 8%).

- Making CBB a relatively important disease requiring immediate attention through development and deployment of resistant varieties.

- Equally, seed companies should consider off-season seed production to produce disease free seed.

Genome-wide association Analysis of Common Bacterial Blight resistance in Andean Gene Pool of common bean

Introduction

- Far way the most important bacterial disease of common bean
- Caused by *Xanthomonas axonopodis* and *Xanthomonas axonopodis* fuscans
- Seed borne, remains in soil and rapidly spreads from leaf to leaf.
- Causes yield losses of up to 60%
- Genetic resistance- most effective control strategy

Intro Cont……

- No single gene has bean found to offer complete resistance
- However, great progress has been made in developing CBB resistant lines such as the VAX lines
- Previous studies have reported over 25 QTLs among which are major and minor.
- SU91, BC420 and SAP 6 – reduces leaf lesions
- GWAS has been instrumental in identifying genes

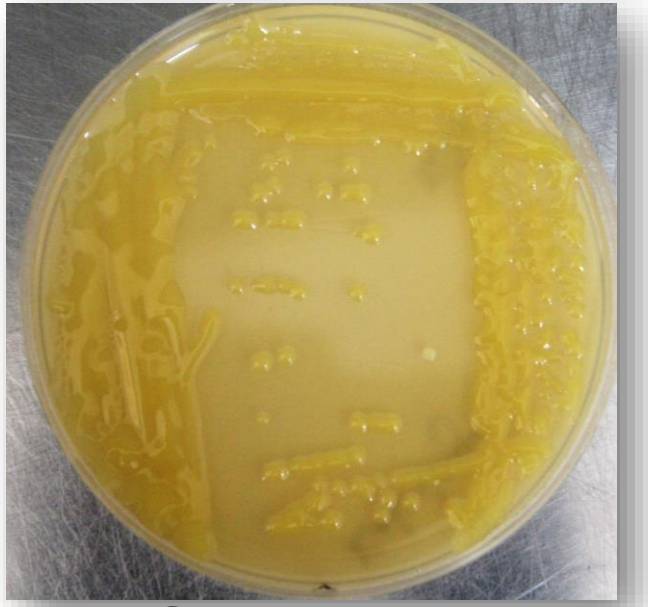
Objective

To identify genomic regions and candidate genes associated with CBB resistance.

Materials and Methods

- 400 ADP lines were screened against 6 strains of CBB (chito, Lusaka,Unza, ZM4, Xa3353 and Xa484A)
- Planted in CRD with 3 reps
- Two plants were inoculated per pot, two leaves each.
- Checks used were; USTP1, USTP5, KAB & LSK

CBB Screening Procedure



Culturing of isolates

Inoculation at 1st trifoliate stage

Scoring 15-20 days after inoculation

Resistant (1-3)

Susceptible (7-9)

CIAT SCALE

- 1-3: Resistant
- 4-6: Moderately resistant
- 7-9: Susceptible

Data analysis

- Disease scores were transformed and analyzed in SAS 9.3 (SAS Institute, 2011).
- The Panel was already genotyped by GBS using 25k SNP markers
- GWAS was conducted in Tassel and Manhattan plots visualized in R
- JBrowse was used to browse the genome for possible candidate genes.

Results and Discussion

Disease Reaction.

- Highly significant differences were observed in all the 6 strains evaluated.

Strain	Total lines	Resistant (1-3)	Moderate (4-6)	Susceptible (7-9)	AVG Score
Xa3353	205	1	15	189	8.6
Xa484A	205	15	10	180	7.7
ZM4	200	10	47	143	6.9
CHITO	404	27	75	302	7.5
LSK	386	29	69	288	7.1
UNZA	137	9	9	119	8.1

Results and Discussion cont…

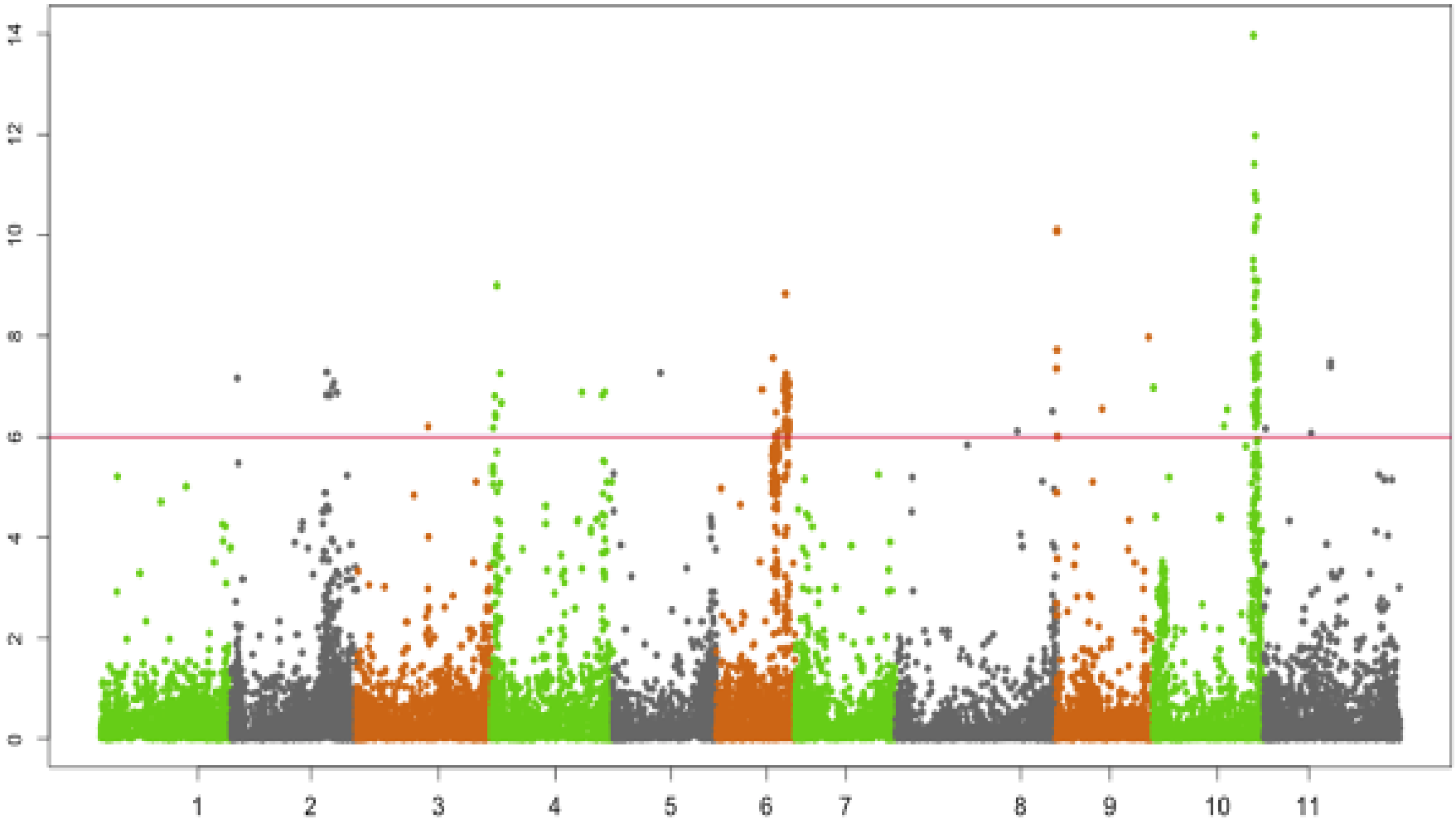
- Only one line ADP 631 was resistant to Xa3353

- Eight genotypes were resistant to 5 strains (ADP17, ADP 525, ADP 632, ADP 583, ADP 733, ADP 756, ADP 118 and ADP 97)

Results and discussion

- 9 Sig SNPs identified across chromosomes; Pv10, Pv9, Pv4, Pv6, Pv11, Pv5,Pv2, Pv3 & Pv8)
- SNP on Pv10 explained **54.8 %** of the observed variation, Pv9 – **45.5**
- 32 possible candidate genes
- **Possible candidate genes-5 on Pv10**
  Phvul.010G119700.1,
  Phvul.010G119700.2,
  Phvul.010G119700.3
  Phvul.010G120500.1
  Phvul.010G120500.2

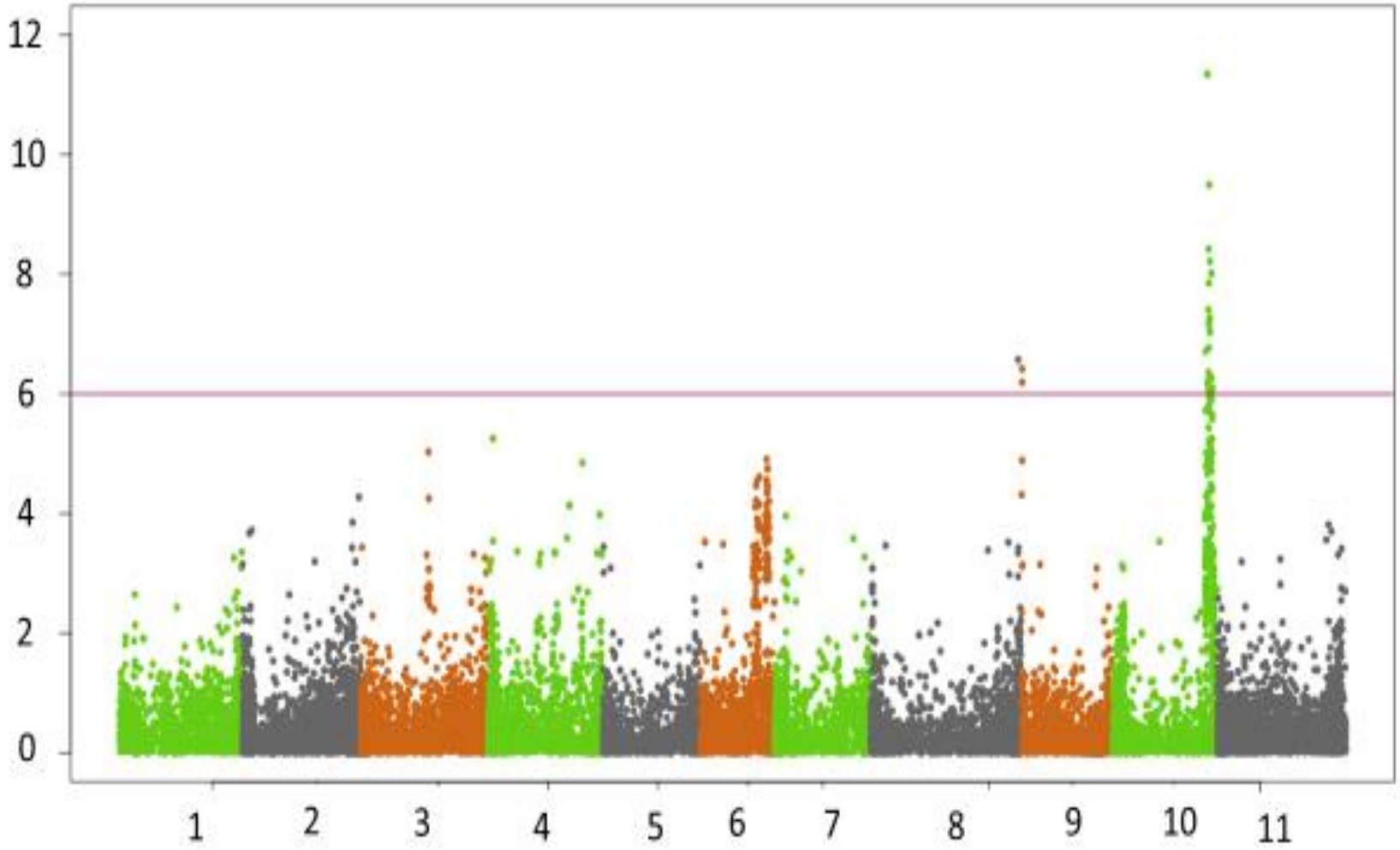
Strain: Xa3353



Results

- 3 Sig SNPs were identified on Pv10, Pv9 & Pv8.
- The SNP on Pv 10 accounted for 38% of the observed variation.
- 10 candidate genes
- **5 possible candidates genes on Pv10**
  Phvul.010G119700.1,
  Phvul.010G119700.2,
  Phvul.010G119700.3
  Phvul.010G120500.1
  Phvul.010G120500.2

Strain: Xa484A

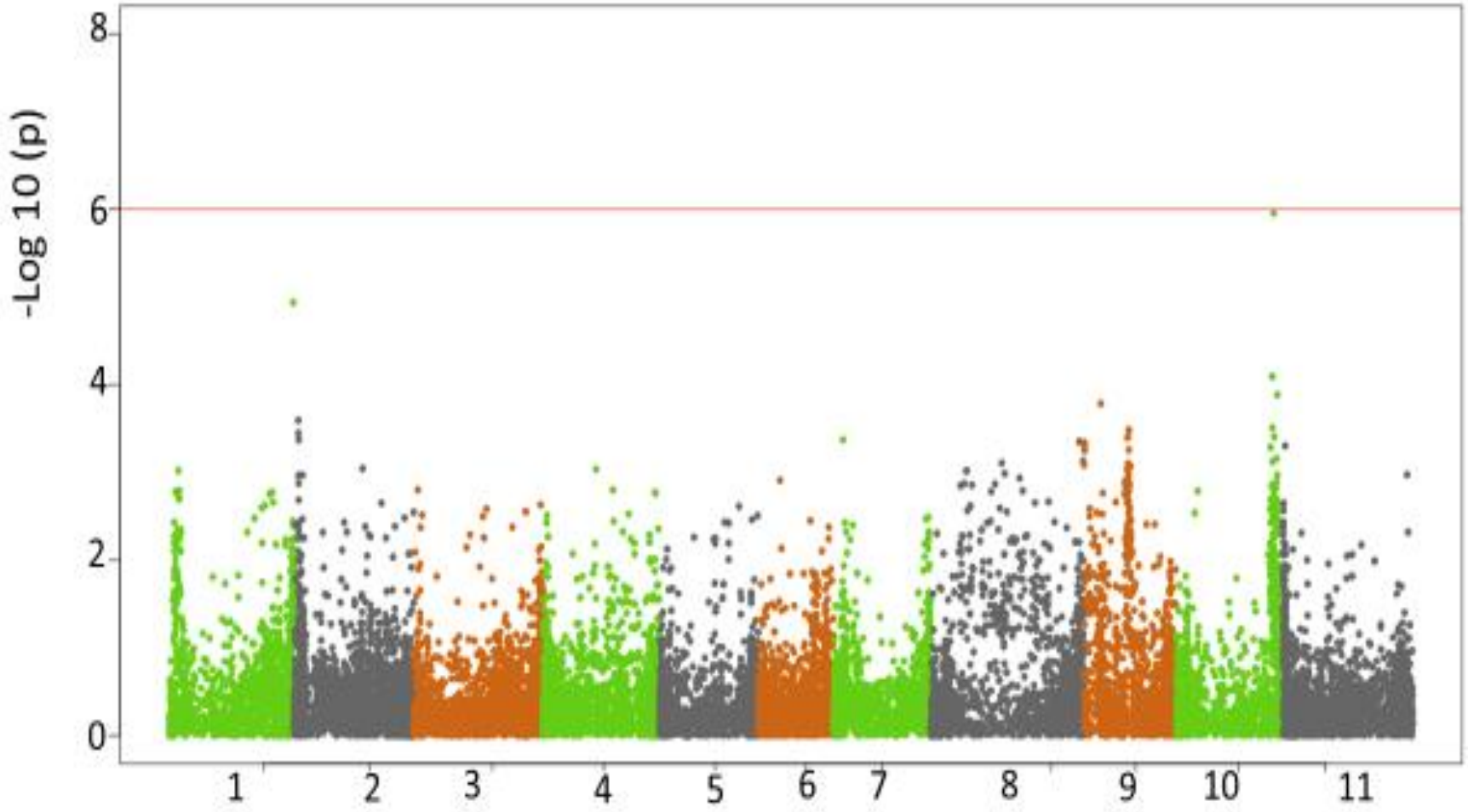


Results

- 1 Sig SNPs was identified on Pv10 explaining 25 % of the variation

- **The SNP was annotated by 5 genes**
  Phvul.010G088500.1,
  Phvul.010G088600.1,
  Phvul.010G090200.1,
  Phvul.010G091100.1,
  Phvul.010G091200.1

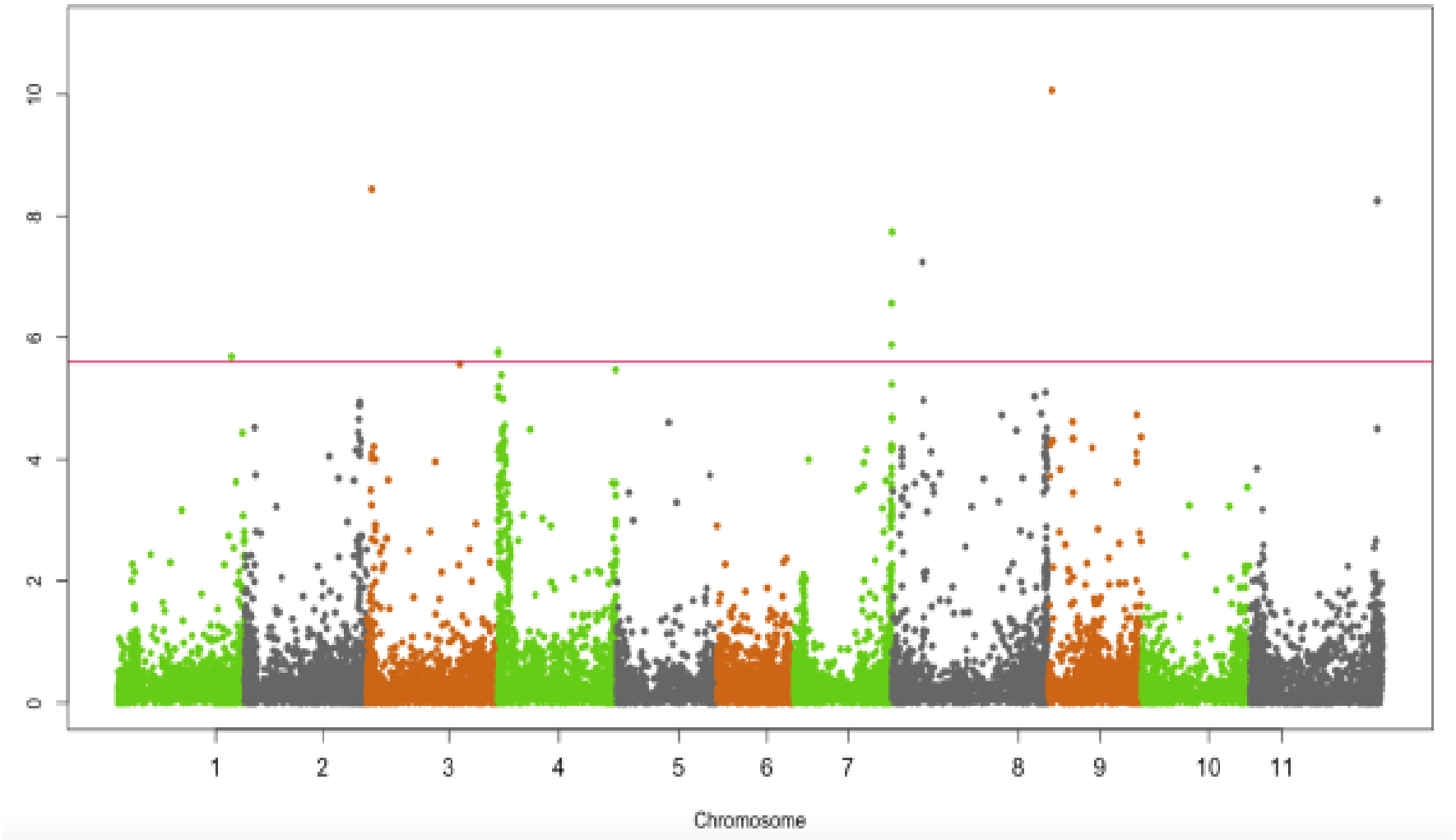
Strain: ZM4



Results

- 5 Sig SNPs were identified on Pv9,Pv11,Pv3,Pv7&Pv8
- Highly sig SNPs on Pv9 explained 24.7% variation.
-
- 39 possible candidate genes were identified
- **5 possible candidates genes on Pv9 were;** Phvul.009G011400.1, Phvul.009G011400.2, Phvul.009G012000.2, Phvul.009G012000.1, Phvul.009G012000.3

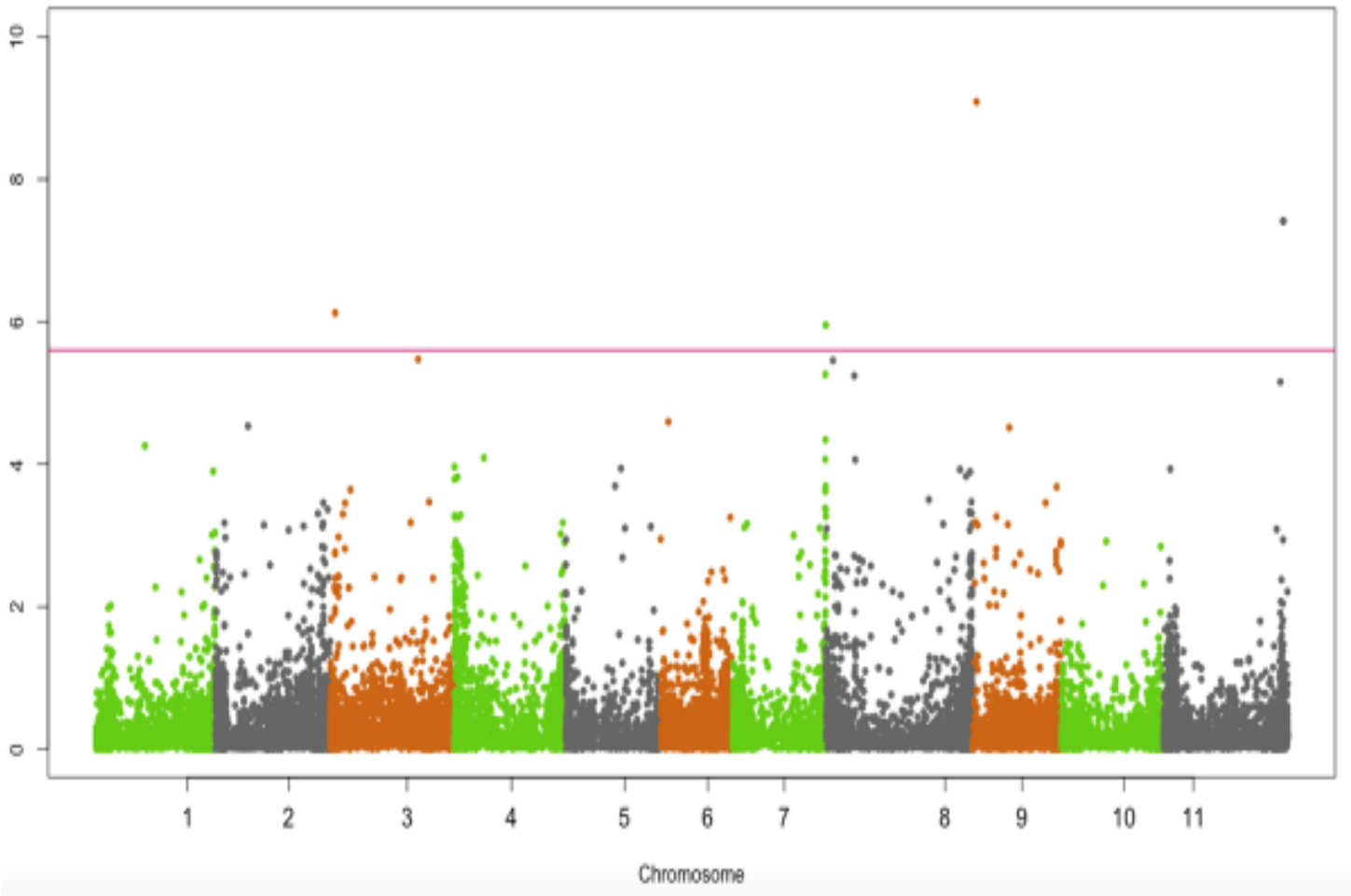
Strain: Chito



Results

- 4 Sig SNPs were identified spread on chromosomes Pv9,Pv11,Pv3 & Pv7
- Highly sig SNPs on Pv9 explained 18.9 % variation.
- **5 possible candidates genes on Pv9 were**; Phvul.009G011400.1, Phvul.009G011400.2, Phvul.009G012000.2, Phvul.009G012000.1, Phvul.009G012000.3

Strain: Lusaka

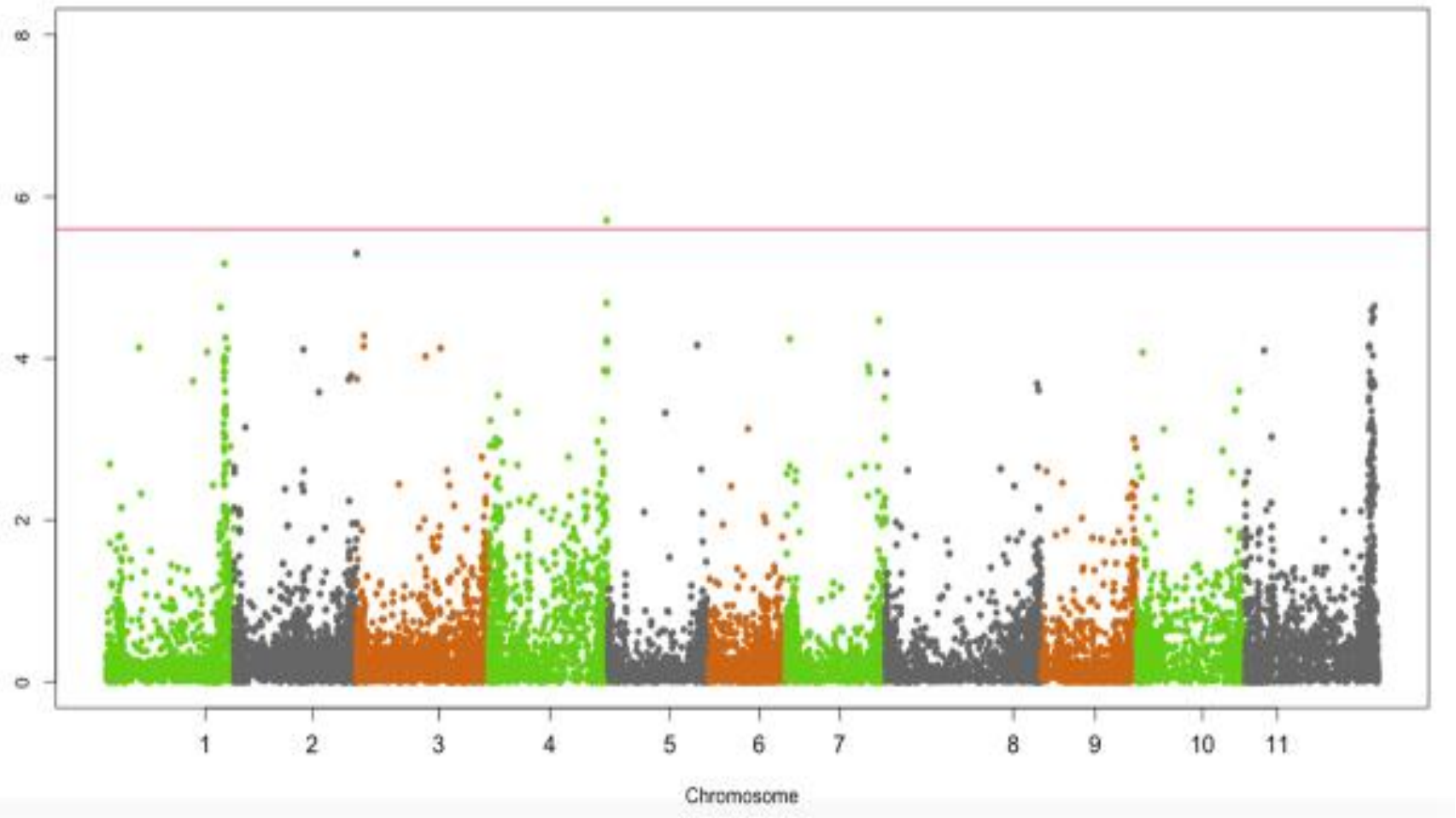


Results

Strain: Unza

- 1 Sig SNPs was identified on chromosomes Pv4 explaining 16.5% variation.

- **26 possible candidates genes identified** Phvul.004G013000.1, Phvul.004G013000.2, Phvul.004G013200.1, etc…



Conclusion

- Resistant genotypes were identified and my be used as cultivar or as parents for CBB resistance
- QTLs conditioning CBB resistance were identified on all the 11 chromosomes.
- Possible candidate genes for CBB resistance were identified
- Breeding programs should therefore, consider pyramiding the identified genes in one background to offer broad spectrum resistance to CBB.
- The QTL on Pv9 should be targeted where strains Xa3353, Xa484A, Chito and Lusaka strains are prevalent.

Update on Objective 3 & 5

Objective 3

- The student will spend 6 months at UC Davis to Genotype and phenotype the population developed from kab and ADP631.

Objective 5

- Seed of selected CBB resistant elite lines has been planted for increase in preparation for a G x E trial next year.

Acknowledgements

Unza Bean Breeding Team

Zari Bean Breeding Team

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