MARKER-ASSISTED PYRAMIDING RESISTANCE GENES AGAINST MAJOR BACTERIAL AND FUNGAL DISEASES INTO COMMON BEAN (*Phaseolus vulgaris* L) VARIETIES WITH FOOD AND MARKET VALUES FOR ETHIOPIA

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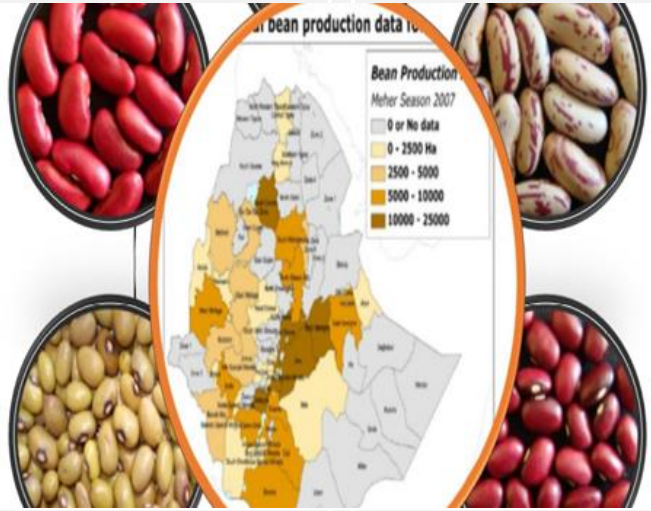
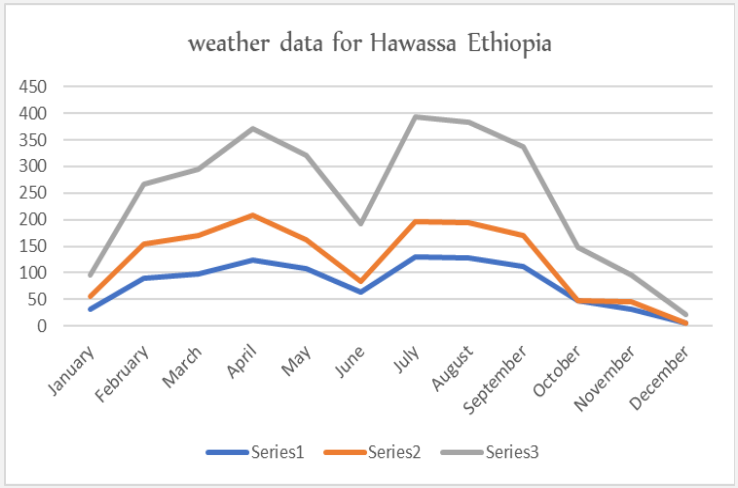
Background

- Common bean (*Phaseolus vulgaris* L.) is the **major pulse** crop grown in the farming systems of Ethiopia,
- For its nutritional, food securities, and economic values.
- With an annual area coverage and production of 350,000 ha & more than 500,000 t with a productivity of 1.5t



The two bean seasons



Ethiopia beans are available most of the year as they follow the diverse climatic conditions of the country. There are at least two seasons that follow rain patterns:

Planting: Belg season-this is the first season that runs from February to May. Second season: the season runs from July to October.

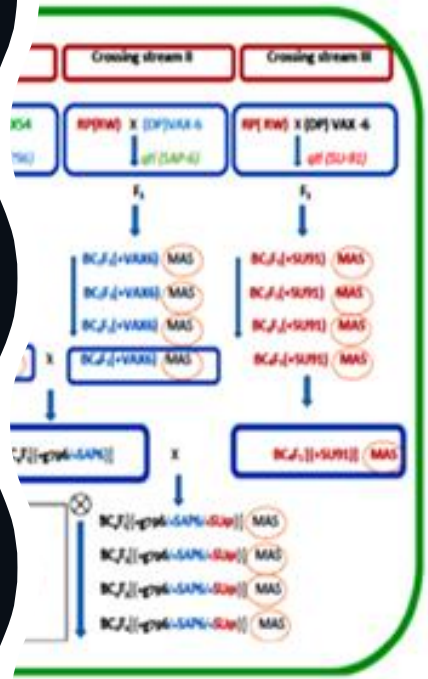
Bean production challenges

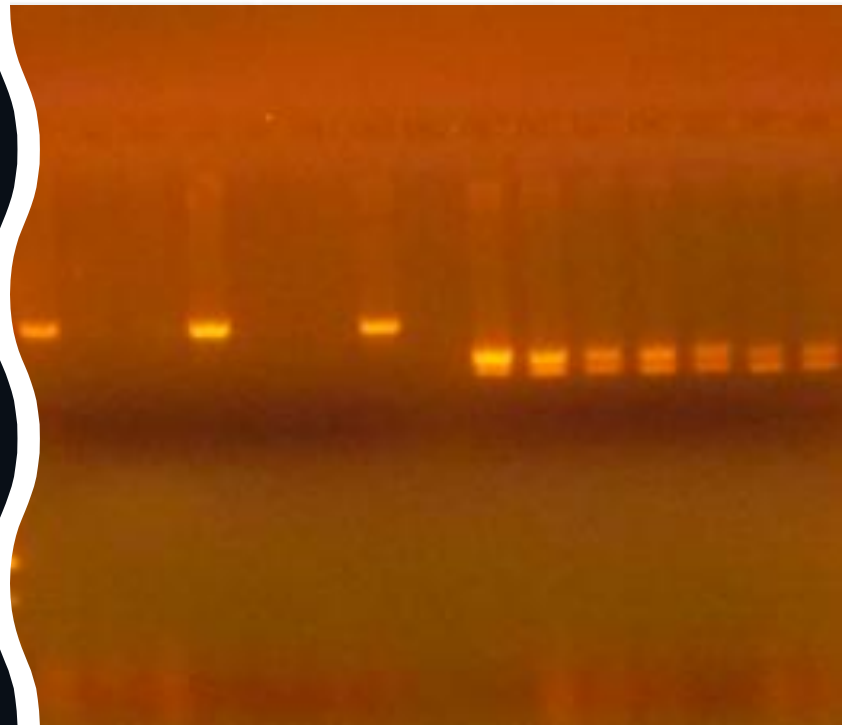
Diseases specifically *angular leaf spot, common bacterial blight, anthracnose, rust, and halo blight are becoming* among the most biotic factors limiting the production of common bean in southern Ethiopia (*affecting 40-100% yield reduction*)

The objective of the Project

- ➔ To deploy disease resistance genes in adapted varieties
- ➔ marker assisted pyramiding R genes into HD & RM bean varieties
- ➔ Prevalce & Charcterization of bean rust & haloblight
- ➔ Capacity building (both physical & training of post grad uate student

Deploying CBB and ALS resistance in adapted varieties of common bean (RW & IBADO)





Materials & Methods

Donor parents

- VAX6 (for CBB R)
- MEX54 (for ALS R)

Recurrent parents

- Redwoliata (Meso)
- Ibado (Andean)

Developed lines

- 15 backcross lines with RW background &
- 19 backcross lines from the IB background

Molecular markers

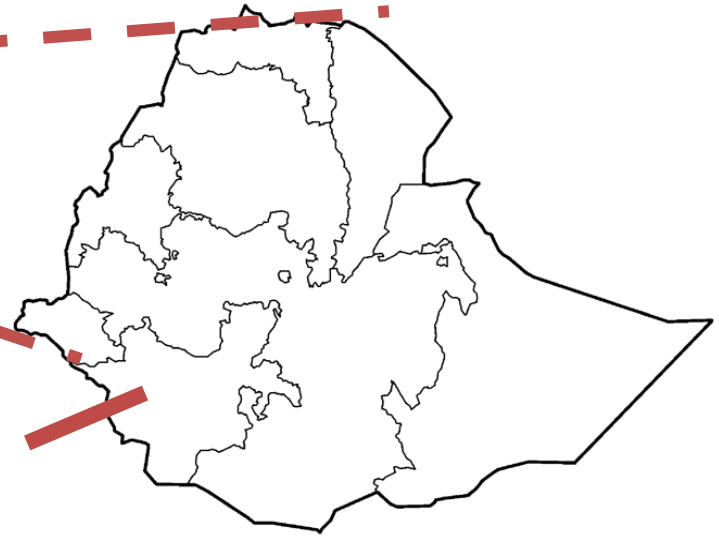
- SAP6 linked to CBBRqtl
- SU91 linked to *CBB Rqtl*
- g796 linked to *Phg-2*

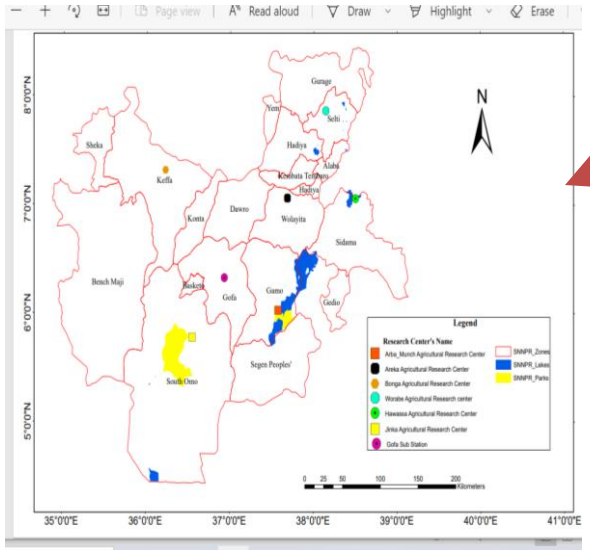
Breeding method

- Parallel backcross

After developing the introgressed lines,

- Advanced back cross breeding lines were evaluated during 2019 - 2020 cropping season under multi-location





Research Center Name	Latitude	Longitude	Altitude (m)
Arba Minch	6.033658°	37.576665°	1203
Areka	7.065964°	37.689099°	1792
Bonga	7.324196°	36.228893°	1733
Hawassa	7.063222°	38.503804°	1714
Jinka	5.783666°	36.553117°	1400
Worabe	7.872376°	38.145228°	2312
Gofa Substation	6.333056°	36.93556°	1266

Map & Geographic locations of the test environment

BC breeding lines with IB & RW background

- The top-yielding BC lines with a high level of resistance to CBB & ALS include:
- **KTABC-I36-3-B; (with RW background)**
- **KTABC-IB-167 (with IB background)**

Seed multiplication for multi-location VVT

Table 1 Amount of candidate & parental lines seed multiplication for VVT trials

No	Candidates & Parents	seed required (kg)	Seed Multiplied (Kg)	Remarks
1	KTABC-IB-167-A	25.0	32.5	Candidate
2	KTABC-IB-170-A	25.0	33.0	Candidate
3	IBADO	25.0	40.0	Check
4	KTABC-05-3	20.0	26.0	Candidate
5	KTABC-05-4	20.0	27.3	Candidate
6	KTABC-06-2	20.0	23.0	Candidate
7	Red wolaita	20.0	22.8	Check

Evaluation of selected backcross lines under on-farm & on-station for (VVT) during 2022 Belge season

Plant materials used

At least one backcrosses developed & selected breeding lines from the previous MAS with RW background as a candidate + 2 checks

At least one backcrosses developed & selected breeding lines from the MAS with IB background as a candidate + 2 checks

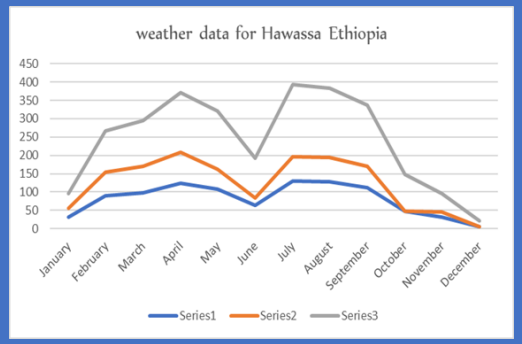
Experimental procedure

- Two candidates' BC breeding lines from the MAS including recent checks have been evaluated in a large plot of 10m x 10m with 2 Reps at the farmers' field
- Locations: Awassa, Gofa & Areka
- Spacing 40cm b/n rows and 10cm b/n plants
- Net plot size: 10m x 10m=100^2
- With recommended agronomic magment practices

Data to be considered during the evaluation

- Days to 50% F
- Days to 90% M
- Seed per pod
- Pod per plant
- 100 seed (g)wt
- Disease ALS & CBB
- Seed YLD (gm/plot)
- Seed color
- Famers preference

VVT on-farm trials @Areka & Gofa area during the first rainy seasons

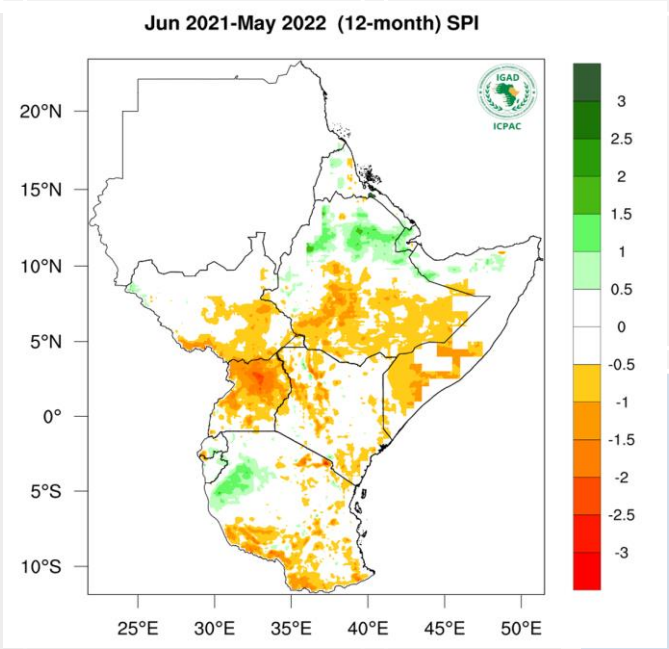


The performance of VVT on-research station & farmers' field @ Areka area

Challenges during the *Belg* season 2022

a moderate to severe multi-season drought conditions in the region (WMO, 2022)

- *Although we have managed to plant our VVTs across all locations including farmers' fields during the Belg season,*
- *we were not fortunate to evaluate our candidates during the season because of the severe moisture deficits due to climate change*
- ***//early drought @ Hawassa & Terminal drought @ Gofa//***



Verification trial at Awassa (2022)

In Ethiopia, bean production is totally rainfed hence our VVT @ Awassa with climate variability (especially low moisture stress) due to a shortage of rainfall (with poor amount and distribution) during the critical growth period After plant emergence @ Awassa & at flowering @ Gofa

VVT on-farm field & station @ Awassa

- The VVT which has been executed at Awassa both on stations & on farmers' fields during the 2022 Belge season has been lost due to low moisture./drought/
- The major breeding objective that needs to be included in our improvement program /currently we are developing materials for such a harsh environment, magic

During the main seasons, managed to plant the VVT's & able to evaluate with the technical committee from the NVRC

1. ***Variety verification trials with small red bean RW background***

1. ***Variety verification trials with large red speckled IBADO background***

at Areka, Gofa, and Hawassa /on-farm & on-stations/

candidate lines developed from the MAS were evaluated across multi-location, visited by NVR the technical committee **Hawassa Gofa & Areka stations**

VVT-small red

- *Back cross lines developed with the MAS that were evaluated across multi-location,*
- *Standard checks with susceptible to cbb @ Areka stations,, the candidates performed & with high level of resistance to CBB & ALS compared to checks under field conditions*

Objective 2: marker-assisted pyramiding R genes into HD & RM bean varieties

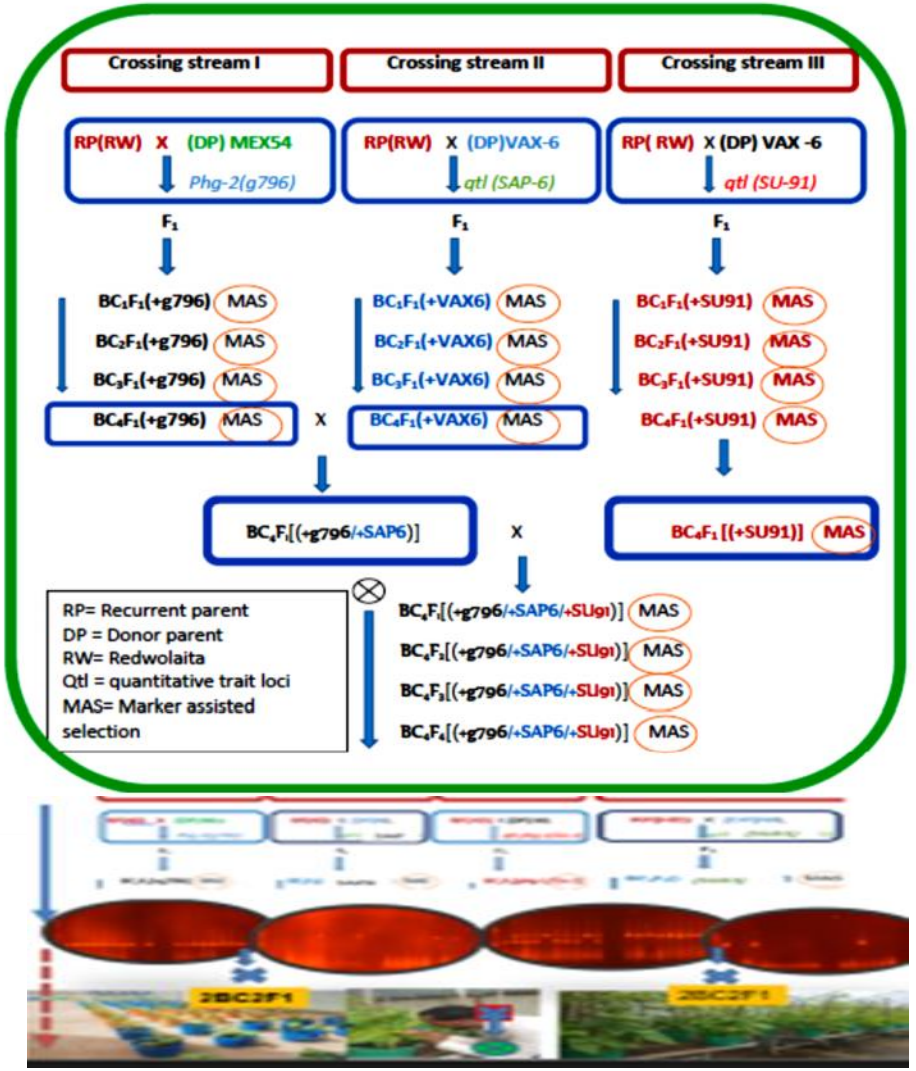
activity 1. Pyramiding **HawassaDume** (released 2007) and **Remeda (**2014) bean varieties through marker-assisted gene pyramiding

Activity 2 virulence characterization *Clletetotricum lindemuthianum* of bean anthracnose

Activity 3 Evaluate the performance of developed lines

Activity 4 Variety verification

MAP-Breeding scheme



Nursery trial MAGP for the HD & RM seed types

- Two sets of (47 HD type & 66 RM type) pyramided lines developed with the MAGP project were evaluated
- Locations: Gofa for one season
- Spacing 40cm b/n rows and 10cm b/n plants
- Design: augmented design

Advanced pyramided RM seed type lines for further MTE evaluation

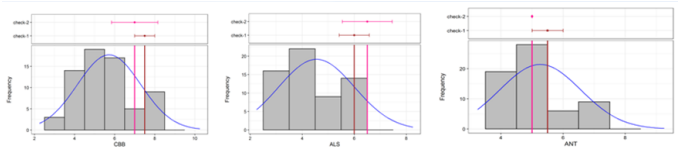


Fig2: Frequency distribution of ALS, CBB & ANT

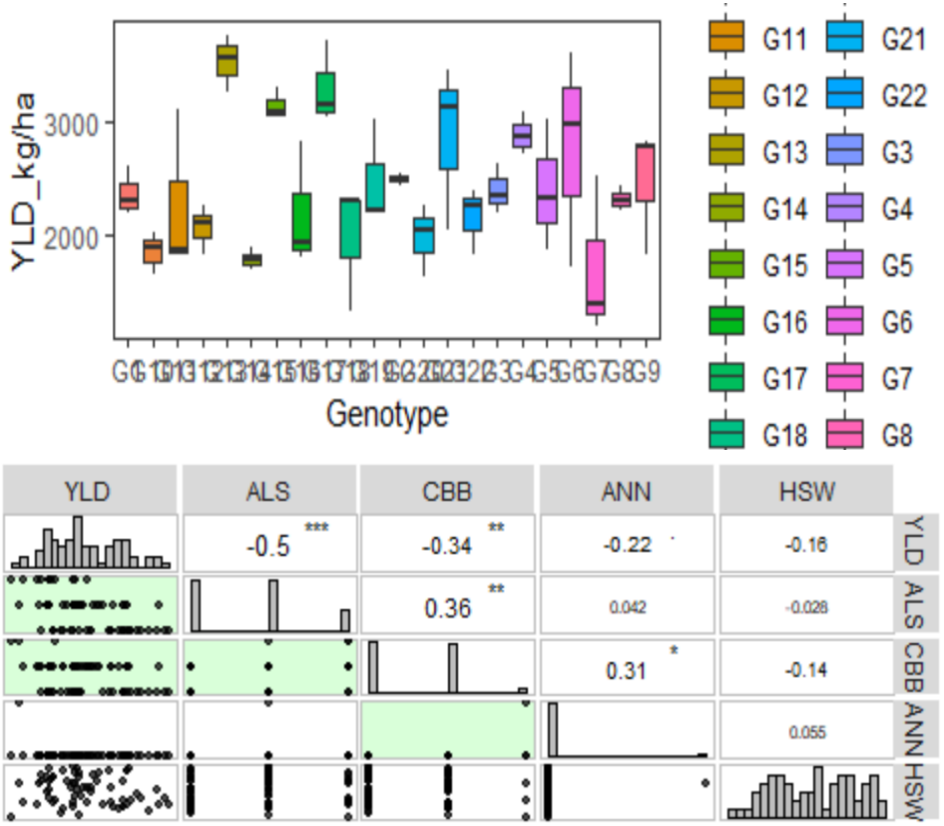
- A total of 68 back cross lines developed from the MAGP project with RM seed type (background) were evaluated under nursery for one season at Gofa research station. (Augmented design + checks)
- 20 BC lines that combined higher yield + multiple resistance (ALS, CBB & Ant) + preferred seed type were advanced to the multi-location evaluation

Table 5 Table 2: Advanced introgressed lines that combined disease resistance & Important agronomic trait with Redmeda seed type & background to be tested across multi-environments

Treatment	Marker	ALS	ANT	CBB	DF	DM	YKPH	selected
KTRM-010-2	sap6	3.25	5.25	6.25	40	85.25	2223.25	1
KTRM-047-1	g796	4.25	4.25	6.25	35	82.75	2258.50	2
KTRM-040-5	g796/sap6/cv	4.25	4.25	6.25	37	85.75	2314.75	3
KTRM-032-6	g796/sap6/cv/su91	3.25	5.25	5.25	34.5	84.75	2341.00	4
KTRM-047-5	g796	4.25	4.25	3.25	34.5	82.75	2397.25	5
KTRM-040-10	g796	5.25	4.25	6.25	38	86.75	2427.25	6
KTRM-047-8	g796	6.25	3.25	5.25	35.5	83.75	2453.50	7
KTRM-032-3	g796/sap6/cv/su91	2.25	5.25	5.25	32.5	83.75	2509.75	8
KTRM-032-5	g796/sap6/cv	3.25	4.25	5.25	33.5	83.75	2509.75	9
KTRM-032-7	g796	2.25	5.25	7.25	32.5	84.75	2509.75	10
KTRM-032-2	g796/sap6/cv	2.25	6.25	5.25	33.5	84.75	2566.00	11
KTRM-002-8	sap6/cv	4.25	3.25	6.25	41	85.25	2617.00	12
KTRM-010-5	g786	3.25	5.25	8.25	40	85.25	2673.25	13
KTRM-002-1	sap6/cv	3.25	4.25	4.25	41	85.25	2729.50	14
KTRM-024-8	sap6	5.25	3.25	5.25	36	82.75	2764.75	15
KTRM-047-9	g796/sap6	6.25	4.25	2.25	33.5	82.75	2791.00	16
KTRM-024-2	sap6	3.25	5.25	5.25	36.5	106.25	2796.00	17
KTRM-002-6	g796/sap6	3.25	5.25	6.25	42	86.25	2898.25	18
KTRM-017-8	sap6	4.25	7.25	6.25	39.5	106.25	2964.75	19
KTRM-040-4	sap6/cv	4.25	4.25	6.25	36	85.75	3046.00	20

Performance evaluation of PLs with RM background at Gofa during the main season, 2022

GEN	YLD	ALS	CBB	ANN	HSW
G1	2366.76	4.3	5.0	3.0	28.9
G10	1848.51	3.7	3.7	3.0	29.7
G11	2263.81	5.7	4.3	3.0	37.0
G12	2060.98	5.7	4.3	3.0	33.2
G13	3541.75	3.0	3.0	3.0	27.4
G14	1785.03	5.0	4.3	3.0	30.1
G15	3154.07	3.0	3.0	3.0	30.3
G16	2181.08	5.0	5.0	3.0	28.7
G17	3311.82	3.0	3.0	3.0	28.8
G18	1976.59	5.0	5.0	3.7	30.3
G19	2485.59	5.7	4.3	3.0	27.0
G2	2493.34	3.7	5.0	3.0	32.1
G20	1977.36	6.3	4.3	3.0	27.8
G21	2878.86	3.0	4.3	3.0	32.9
G22	2154.82	5.0	4.3	3.0	29.5
G3	2389.67	4.3	3.7	3.0	31.9
G4	2891.68	5.0	3.7	3.0	23.7
G5	2408.02	4.3	3.0	3.0	31.1
G6	2769.69	3.7	4.3	3.0	26.9
G7	1701.36	5.7	5.7	3.0	24.9
G8	2320.54	5.0	3.7	3.0	33.8
G9	2474.56	4.3	3.7	3.0	31.8
Mean	2248.91	4.5	4.5	4.1	29.9
LSD	**	*	ns	ns	ns
CV	19.3	26.5	26.5	27.1	19.8



Advanced pyramided HD seed type lines for further MTE evaluation

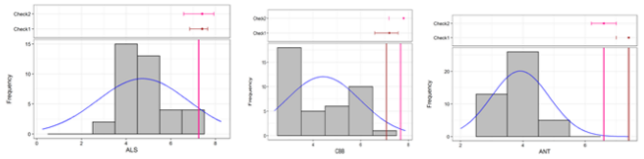


Fig1: Frequency distribution of ALS, CBB & ANT

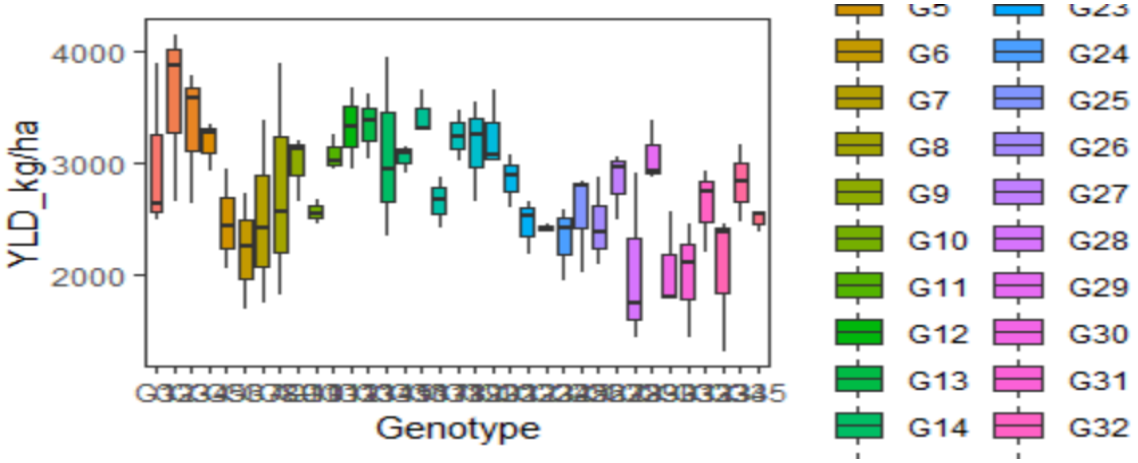
- A total of 47 back cross lines developed from the MAGP project with HD seed type (background) were evaluated under nursery for one season at Gofa research station.
- 33 BC lines that combined higher yield + multiple resistance (ALS, CBB & Ant) + preferred seed type were advanced to the multi-location evaluation

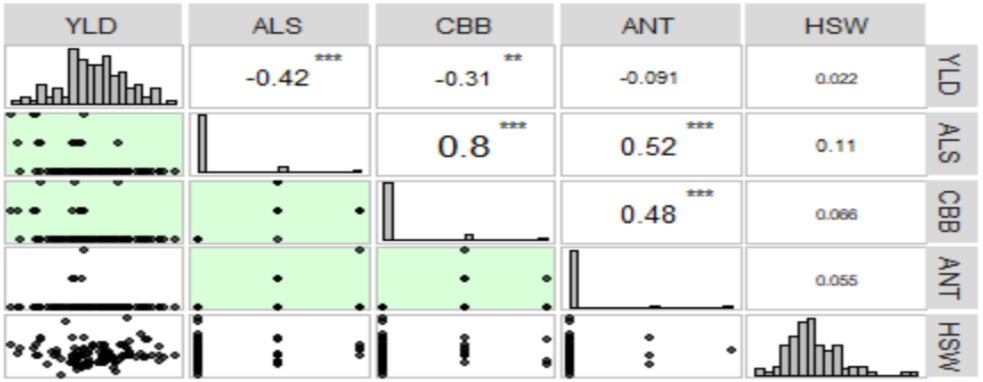
Table 3: Advanced introgressed lines that combined disease resistance & Important agronomic trait with Hawassdume seed type & background to be tested across multi-environments

	Treatment	Markers/introgression	ALS	ANT	CBB	DF	DM	YKPH
1	KTHD-253-9	sap6/cv54	4.25	3	3.37	38.1	78.12	2395.25
2	KTHD-015-1	sap6/cv54/g796/su91	6.75	3	4.87	32.1	75.12	2658.25
3	KTHD-015-4	sap6/cv54/g796/su97	3.75	4	4.87	33.1	76.12	2546.25
4	KTHD-015-5	sap6/cv54/g796/su94	3.75	4	3.87	34.1	76.12	3162.25
5	KTHD-015-7	sap6/cv54/g796/su97	4.75	5	3.87	33.1	76.12	2602.25
6	KTHD-064-1	cv54/g796	3.75	3	7.88	32.1	76.12	2442.25
7	KTHD-064-4	cv54/g796	0.25	3	6.37	34.1	76.62	3218.25
8	KTHD-223-2	g796/su91	4.75	4	3.38	38.6	84.13	2496.25
9	KTHD-223-3	g796/su91	3.75	4	3.38	38.6	84.13	2776.25
10	KTHD-223-4	g796/su91	4.75	4	3.37	39.6	84.12	3224.25
11	KTHD-223-5	g796/su91	4.75	4	3.38	39.6	84.12	3392.25
12	KTHD-230-2	g796/su91	3.75	4	3.38	38.6	83.13	2616.25
13	KTHD-230-3	g796/su91	4.75	4	3.38	39.6	84.13	2896.25
14	KTHD-230-4	g796/su91	4.75	4	3.38	39.6	84.13	2896.25
15	KTHD-230-5	g796/su91	4.75	4	3.38	38.6	82.13	2448.25
16	KTHD-238-2	g796/su91	4.75	4	3.38	36.6	82.13	2888.25
17	KTHD-245-3	g796/su91	8.25	4	6.38	37.1	77.12	2835.25
18	KTHD-245-5	g796/su91	8.25	4	6.37	39.1	77.12	2603.25
19	KTHD-245-7	g796/su91	8.25	4	6.37	38.1	78.12	2835.25
20	KTHD-253-6	sap6/cv54	4.25	3	2.37	38.1	78.12	2675.25
21	KTHD-253-8	sap6/cv54	5.25	3	3.37	39.1	79.12	3611.25
22	KTHD-276-1	sap6	6.25	3	3.37	36.1	75.62	2490.25
23	KTHD-276-3	sap6	6.25	5	3.37	38.1	75.62	2562.25
24	KTHD-276-4	sap6	6.25	5	3.37	38.1	75.62	2714.25

Performance evaluation of PLs with HD background at Gofa during the main season, 2022

GEN	YLD_kg/h	ALS	CBB	ANT	HSW
G1	3001.00	3	3	3	20.1
G2	3557.21	3	3	3	19.6
G3	3331.00	3	3	3	20.2
G4	3178.47	3	3	3	21.8
G5	2470.67	3	3	3	19.1
G6	2216.63	3	3	3	23.9
G7	2508.17	4	4	4	20.9
G8	2753.18	3	3	3	21.7
G9	2991.27	3	3	3	19.9
G10	2553.51	3	3	3	21.1
G11	3073.38	3	3	3	21.2
G12	3312.34	3	3	3	23.7
G13	3339.55	3	3	3	20.5
G14	3079.89	3	3	3	20.1
G15	3049.28	3	3	3	20.0
G16	3425.01	3	3	3	20.9
G17	2652.40	3	3	3	21.0
G18	3238.54	3	3	3	20.1
G19	3153.03	3	3	3	21.3
G20	3244.85	3	3	3	21.8
G21	2846.52	3	3	3	19.0
G22	2448.80	3	3	3	17.9
G23	2414.35	3	3	3	19.3
G24	2312.04	3	3	3	20.9
G25	2548.68	3	3	3	21.1
G26	2442.50	3	3	3	20.2
G27	2833.46	3	3	3	19.8
G28	2026.65	3	3	3	20.5
G29	3057.25	3	3	3	21.5
G30	2045.80	3	3	3	20.9
G31	1991.43	3	3	3	20.5
G32	2615.60	3	3	3	21.6
G33	2042.40	3	3	3	21.5
G34	2824.60	4	4	3	20.3
G35	3160.20	6	5	6	22.0
Mean	2792.56	3.1	3.1	2.1	20.7
LSD	279.5	0.5	0.9	0.5	8.5
CV	17.5	11.1	17.1	8.9	2.8





Current SARI ABC project

3. Objectives

3.1. Long-term objective

- To enhance common bean (*Phaseolus vagaries* L) production and productivity through development and dissemination of improved bean technologies that contribute to food security, nutrition, income generation and resilience to environmental stresses in Ethiopia

3.2. Specific objectives

- Develop multiple disease resistance, high yielding & farmer preferred bean varieties suitable for different cropping systems with MAS techniques
- Pathogen variability, prevalence & geographic distribution of major bean disease of halo blight (HB) & bean RUST
- Capacity development both human and physical (training of scientist in modern molecular marker technology in relation to molecular breeding in bean improvement)

Introduction & seed multiplication of bean rust & halo blight sets of differential sets

#	Rust diffrentials	#	Haloblight differntials
1	RUST -RED LAND PIONEER	1	Tender grain)
2	RUST -CURO NEGRO	2	Canadian wonder)
3	RUST -G6416	3	Edmund PV 86061)
4	RUST -MEXICO 235	4	Red Mexico 4T3)
5	RUST - CMC	5	Gutemala 196B)
6	RUST -G 5719	6	A52 (2AA54)
7	RUST -PI 181996	7	A53 (2AA55)
8	RUST -GM 1140	8	A43 (2AA12)
9	RUST - MEXICO 309		
10	EARLY GALATIN		

Table 3. Set of 12 common bean differential cultivars proposed at the Third International Bean Rust Workshop held in South Africa in 2002 and used in the characterization of the virulence diversity of *Uromyces appendiculatus*

	Bean Differential Cultivars[1]	Rust R Gene	Gene Pool[2]	Binary Value[3]	Linkage Group where rust resistance gene is mapped
	Andean Beans				
1	Early Gallatin	*Ur-4*	A/MA	1	Pv 06
2	Redlands Pioneer	*Ur-13*	A	2	Pv 08
3	Montcalm	Unknown	A	4	
4	PC 50	*Ur-9, Ur-12*	A	8	Pv 01 (*Ur-9*), Pv 7 (*Ur-12*)
5	Golden Gate Wax	*Ur-6*	A/MA	16	Pv 11
6	PI 260418	Unknown	A	32	
	Middle American Beans				
7	GN 1140	*Ur-7*	MA	1	Pv 11
8	Aurora	*Ur-3*	MA	2	Pv 11
9	Mexico 309	*Ur-5*	MA	4	Pv 04
10	Mexico 235	*Ur-3+*	MA	8	
11	CNC	Unknown	MA	16	
12	PI 181996	*Ur-11*	MA	32	Pv 11

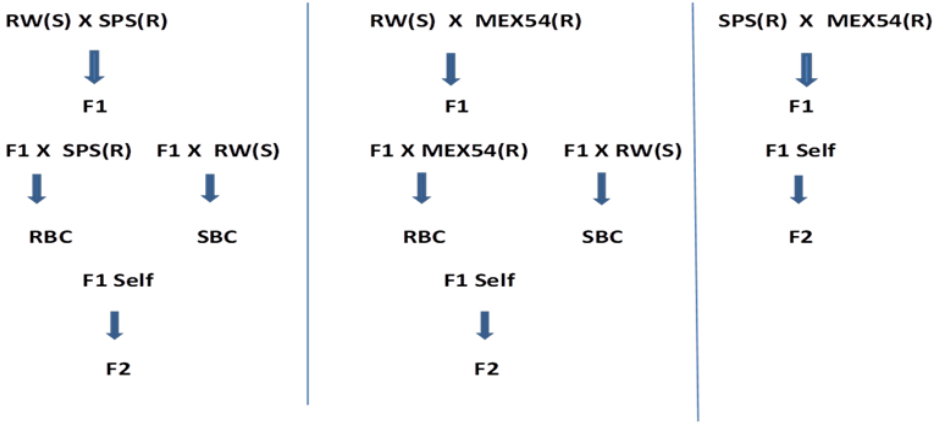
[1]PC 50 = Pompadour Checa 50; GN 1140 = Great Northern 1140; CNC = Compuesto Negro Chimaltenango.
[2]Gene pool, origin, of bean cultivars: A = Andean, MA=Middle American
[3]Example of how races of the bean rust pathogen are named using this set of six Andean and six Middle American differential cultivars and the binary system. If a new isolate of the

objective 4 capacity building

One molecular lab & screening house were equipped for employing marker-assisted breeding activities

So far, Five post-graduate students were granted for their studentship by KT

Physical capacity for employing marker-assisted breeding activities in common beans for Ethiopia



#	Student	Study topic		University
1	**Tigist Firew**	Genetic Characterization of angular leaf spot resistance in selected common bean (*Phaseolus Vulgaris* L.(cultivar in Ethiopia		Hawassa University
2	**Yisahak Tsegaye**	Survey and virulence characterization of the *Uromyces appendiculatus* , the cause of common bean rust, in major bean growing areas of Southern Ethiopia		Hawassa University

postgraduate students

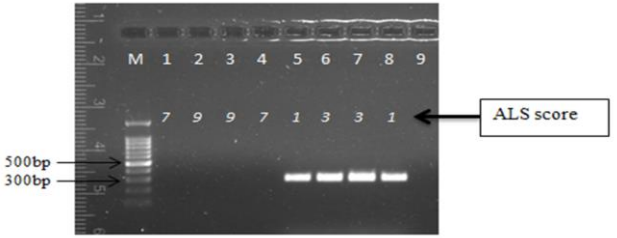
***Tigist F.**, an MSc student working at lab genotyping developed pops to characterize the inheritance and allelic relations of resistance in **SPS50H** bean genotypes compared to resistance in Mex-54*

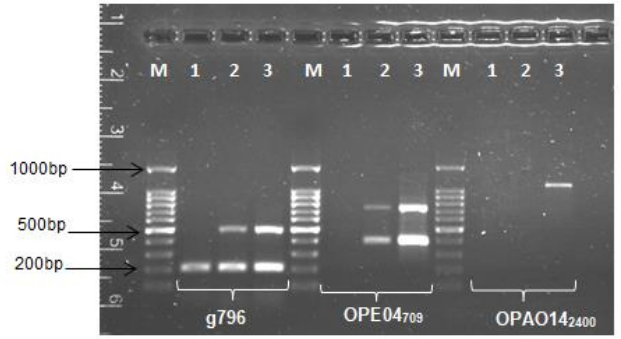
The finding from Tigist's research

- The two study populations were developed by crossing resistance parent SPS50HB with susceptible parent RW and resistance parent SPS50HB with Mexico 54 (RXR).

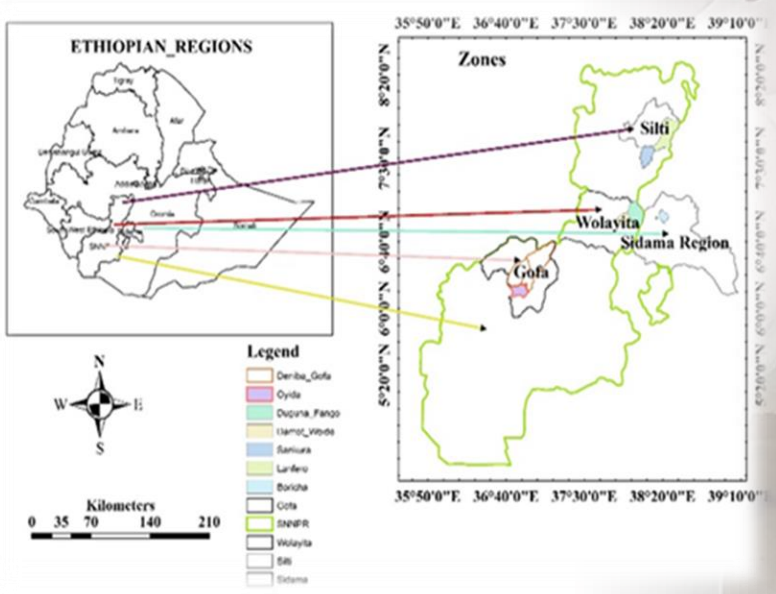
- F2 and SBC populations were evaluated for their reaction to ALS and the chi-square test result shows that SPS50HB has the resistance gene that is inherited as two independent dominant genes.
- *Co-segregation analysis of the polymorphic marker and disease reaction in the F2 population derived from RW x SPS50HB showed that these two markers (OPAO4$_{709}$ and PF13$_{310}$) were associated with resistance to ALS in SPS50HB and F2 individuals*

- The F2 populations from the R X R show no segregation for the angular leaf spot, this indicating the resistance gene in SPS50HB and Mexico 54 genotypes ,co-segregate and is either in the same locus or are closely linked genes.

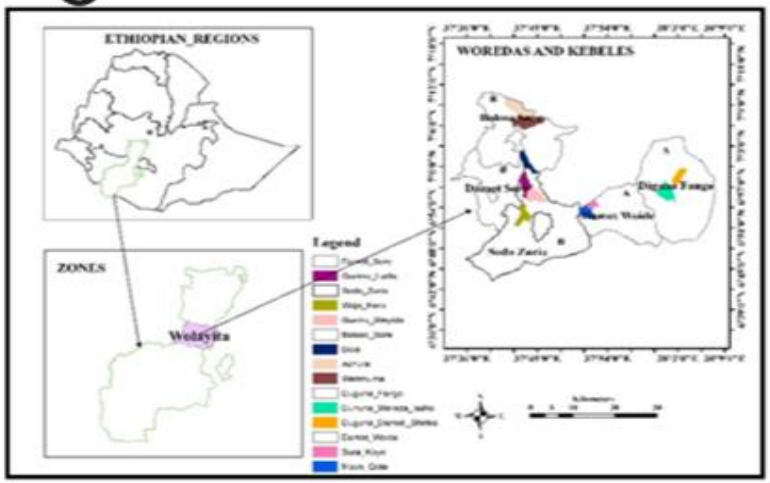


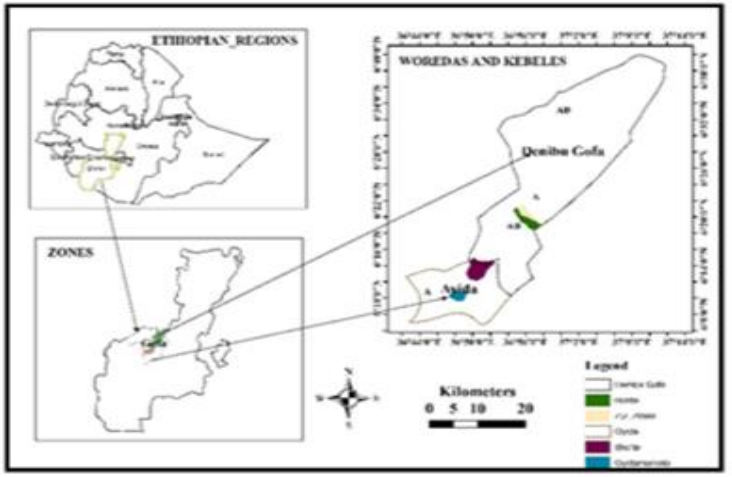


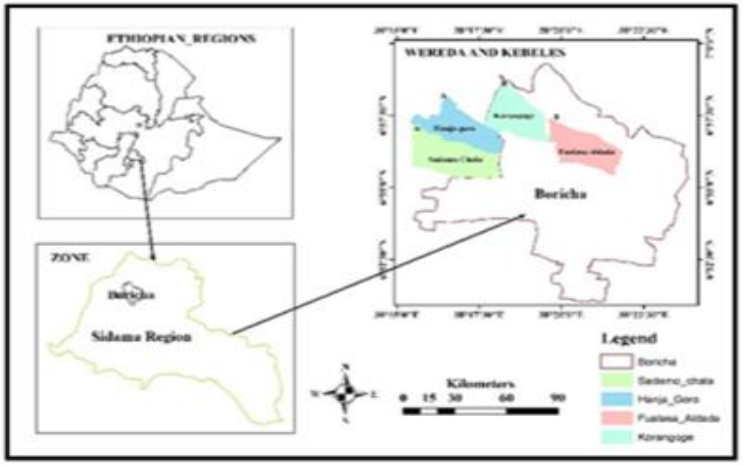
***Yisahak T.**, an MSc student evaluating the virulence of the* *Uromyces appendiculatus* *of the bean rust pathogen collected from the bean growing areas of southern Ethiopia*

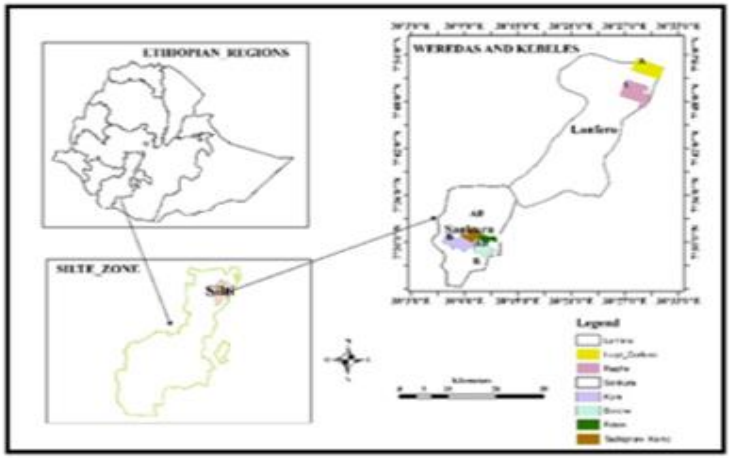


after collecting *uredospore of bean rust, he is currently evaluating and characterizing the virulence with susceptible and sets differentials*









Note: *with letter A are rust surveyed area, with letter B are halow blight surveyed areas and with letter AB is for both rust and halow blight*

Progress in a molecular lab set-up @ SARI Ethiopia



Current Molecular lab @ SARI, Ethiopia

Our lab & activities has been visited by graduate students from the local universities visiting

Recently the standing committee of agriculture & Representative of the house of the federation of the **EFDRF** visited the lab & our activities

The way forwarded

- Breeder seed multiplication of the new varieties
- Evaluate the advanced PLS lines of **HD & RM bean types** under multi-location trial & select the best candidates for the next VVT,
- Finalize the survey & prevalence study & characterization of **bean rust & HB** pathogens from the major bean production area
- Continue crossing targeting bean rust, anthracnose, and bean halo blight & Continue MAS breeding activity for ANT, RUST & HB resistance in preferred bean cultivars /**HD & Dinkinesh**/
- Integrate drought resistance in our breeding program

KT ABC annual meeting @ Hawassa, Ethiopia 2014 hosted by SARI

More than 40 participants from different countries

Acknowledgements

Kirkhouse Trust
Supporting research and education in the biological sciences

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Thank you for listening