

# Disease screening in Phaseolus hybrids



- Plants growing in the soil glasshouse at NIAB Cambridge.
- Jute webbing to support the plants on wires.
- Drip irrigation.
- Temperature maintained at approx. 12-20°C with supplemental light.
- 30 plants per genotype in 3 reps, 10 plants per plot.
- Resistant and susceptible control plants included for each disease.

Alliance



## Disease screening programme







Sclerotinia (white mold) – widespread and severe.

Rhizoctonia Solani – encouraged by warming climate. Foliar and Root infecting forms.

Colletotrichum (Anthracnose) widespread and severe.

Disease	Planting location	Assay	Inoculation
Sclerotinia	Glasshouse	Detached leaf	Growth room
Web blight	Glasshouse	Detached leaf	Growth room
Anthracnose	Glasshouse	Detached leaf	Growth room
Root rot	Growth room	Seedling test	Growth room

## Growth room inoculations: Detached leaf

Leaves arranged in corning bioassay trays containing wetted filter paper and plastic mesh.



- Three sclerotinia isolates; wide host range: brassica and legumes; pea, beans, oilseed rape, carrots, lettuce etc. Two
  pea isolates and one Phaseolus sclerotinia (isolate Ph). All originated in the UK.
- Web blight isolates obtained from CIAT, Colombia.
- 5mm mycelial plug from a 5 day old plate transferred to adaxial side of each leaflet just off centre.
- Drop of water added to prevent agar disc drying out.
- Both sclerotinia and web blight isolates grown on PDA.
- Assessments 3-7 dai. Measure lesions size and photograph for image analysis



### Detached leaf assay

- Harvest 3<sup>rd</sup>-5<sup>th</sup> fully expanded leaf.
- Leaves transferred to corning boxes in controlled environment. chambers for inoculations.
- Petiole of each trifoliate leaf makes contact with damp filter paper.
- Sclerotinia inoculated leaves incubated at 22°C day and Web blight 27°C.
- Advantages:
  - Comparing large numbers of individuals
  - Trifoliate leaf allows for three isolates per disease
  - Each plant can be resampled
  - Can be resampled to allow for more than one disease per plant.
  - Non-destructive so can obtain seed for future crossing
- Disadvantage:
  - Disease response may differ in whole plant



#### Disease screening: Sclerotinia sclerotiorum



#### Mild disease symptoms on resistant plants

#### Severe disease symptoms on susceptible plants

### Disease screening: Web blight Rhizoctonia solani.





#### Mild Web blight symptoms

Severe Web blight symptoms

#### White Mold (*Sclerotinia sclerotiorum*) mean score of all three isolates



#### Web blight mean disease both isolates



dumosus x coccineus

### Disease screening: Rhizoctonia Solani root rot

- Two Rhizcoctonia Solani isolates (Colombia) Rh-5 and Rh-17
- Two reps per isolate
- 10 seeds per trays per rep
- Isolates inoculate at a concentration of 1.5% (w/w by soil volume)
- Three resistant controls
- G881 (Mexico)
- G1540 (Sweden)
- G3151 (Guatemala)
- Disease assessed 14-28 days after inoculation (dai)



Disease index		Symptoms around hypocotyl
0	No disease	No symptoms present
1	<5%	Lesion appearing
2	10%	Lesion present <2.5mm
3	25%	Lesions 2.5 to 5mm, plant healthy but disease beginning to penetrate stem
4	50%	Lesions >5mm, disease penetrating stem
5		Disease penetrating stem, some stunting
6	>75%	Disease lesion significant around and within stem
7		Lesions severe, plants stunted
8	>90%,	Withered and dying
9		Plant dead

### Rhizoctonia root and stem lesions assessed around hypocotyl



No Symptoms

Disease index 3

Disease Index 5 Disease Index 7 Disease Index 9

#### 9 8 7 6 Disease Index 5 Δ 3 2 1 0 G40001 Parent G5773 Parent G5773 Parent G52443-2 G52443-9 G52443-9 G52443-9 G52443-9 G52443-9 G52443-10 G52443-1 G52443-1 G23871 Parent G23860M-2 Parent G23860H-13 G23860H-15 G23860H-16 G23860H-10 G23860H-5 G23860H-5 G23860H-4 G23860H-4 G23860H-4 G23860H-4 G35361 Parent G51274D Parent G51274i-7 G51274i-8 G51274i-4 G51274i-2 G51274i-22 G51274i-22 G881 Control G1540 Control G3153 Control G24764B-13 G24764B-11 G24764B-10 G24764B-10 G24764B-8 G35998 G50785v1-12 G50785v1-12 G50785v1-1 G50785v1-4 G50785v1-45 G50785v1-45 G36211 G50879V1 G50879X4-12 G50879X4-6 G35270 parent G35271 parent G36124-1 G36124-15 G36124-14 Ser-16 G24764D parent 2 Ŀ. 2 G24764B-9 G36124-G36124-G36124-G36124-G36124-G36124-Complex 11 Complex 19 Case 10 Complex 14 Complex 3 Complex 7 Complex 8 vulgaris x dumosus x vulgaris x dumosus x Vulgaris feral vulgaris x vulgaris x parvifolius x vulagris coccineus coccineus coccineus coccineus acutifolius

#### Rhizoctonia root rot (mean scores for both isolates Rh-5 & Rh-17)

### Disease screening: Anthracnose

#### Colletotrichum lindemuthianum Isolates

- Detached leaf assay protocol as before.
- Inoculate with a filtered spore suspension at 2 x 10<sup>6</sup>
- Assess 7-10 dai.

Isolate	Origin	Sporulation on PDA	Typical Spore count/ml	Vein symptoms on susceptible control G1149	Media
CL638	Colombia	Yes	5 x 10 <sup>6</sup>	high	PDA
KISO2	Uganda	Yes	1 x 10 <sup>6</sup>	low	MM/PDA
RA-9B	Uganda	Yes	3 x 10 <sup>6</sup>	v.low	MM/PDA
KB011	Uganda	low	<10 <sup>3</sup>	low	MM/PBA







KISO2







RA-9B

KB011



### Anthracnose disease symptoms



#### G2338 Resistant control

G1149 Susceptible control





### G52443-10 Case 10

### G36124-5 Complex 14

#### Anthracnose mean percentage disease CL638





## Summary

Good levels of resistance or tolerance discovered for all diseases screened.

Each accession was also scored for vigor i.e leaf or seedling integrity following disease inoculation to give an overall disease rating for each complex.

Disease profile has now been established for all accessions screened.

Disease profiles of putative parents and hybrids shown to match that observed in situ.

Disease resistance of accessions tested in end-user environment would complement the disease profile from this screening programme.



