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Rating scale for field evaluations of common bean genotypes (accessions, advanced lines, or varieties) using artificial inoculation

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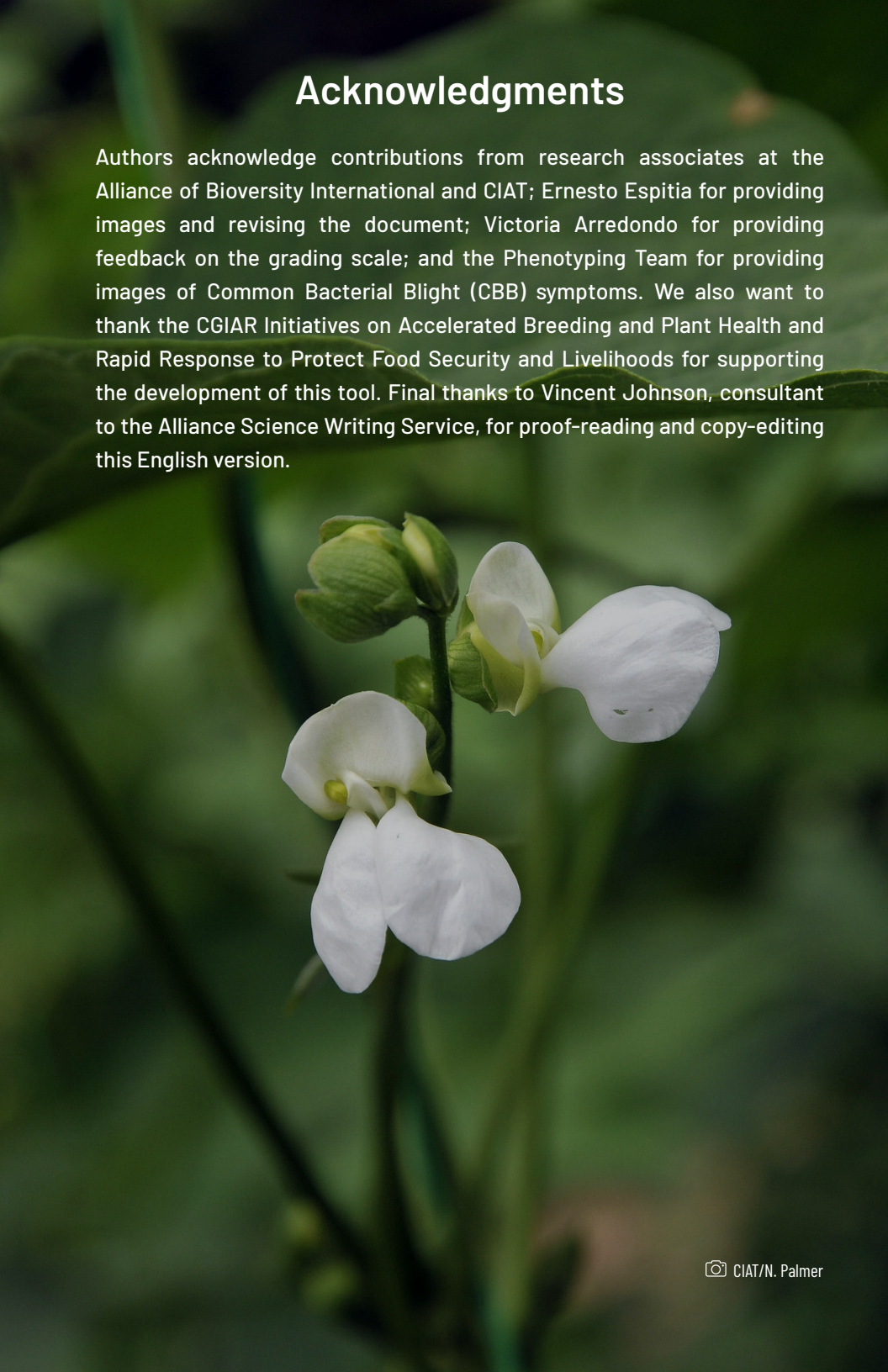

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The information described in the following notes applies to all diseases.

Note 1. The Standard Evaluation System consists of nine categories using whole numbers, from 1 to 9, without decimal places. Category 0 (zero) is not included in the scale, and it may be used to record the absence of disease or the inability to conduct the evaluation due to any particular situation.

Note 2. This scale is designed based on the disease pattern of infection under artificial inoculation conditions.

Note 3. For scores 2 to 9, 30% or more plants in a row or plot must meet the described condition. For the final evaluation, in case the plant's middle and upper parts show leaves with different degrees of damage, the degree of severity must focus on the maximum degree of damage caused by the disease.

Note 4. For the third evaluation, score the degree of severity based on the symptoms observed for the pods.

Note 5. Conduct at least three evaluations to cover plant development from the beginning of the reproductive stage throughout the mid-seed-filling stage.

Note 6. Genotypes with scores 1–3 are regarded as resistant; 4, 5, and 6, as intermediate, and 7–9 as highly susceptible.

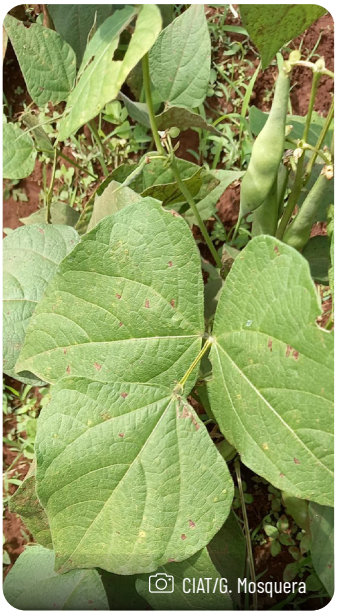
Annex 1 shows a sketch useful for estimating the percentage of leaf area affected by symptoms when using the evaluation scale.

Rating scale for Angular Leaf Spot (*Pseudocercospora griseola*) First evaluation (R5 stages)

Grade 1. No disease symptoms in any part of the plant.

Grade 2. Presence of a few small lesions (< 2 mm) with no sporulation.

Grade 3. Presence of small lesions (< 2 mm) with no sporulation, covering approximately 2% of the leaf area.



Grade 4. Presence of several generally small necrotic angular lesions (≥ 3 mm), limited by the primary and secondary veins on the upper side of leaves, with low sporulation on the underside of the leaves, covering approximately 3% to 4% of the leaf area.

Grade 5. Presence of a higher number of generally medium-sized angular lesions (≥ 4 mm) defined by the veins with low sporulation on the underside of the leaves, covering approximately 5% of the leaf area.

Grade 6. Abundant generally large lesions (≥ 5 mm) with sporulation on the underside of the leaves, covering between 6% and 9% of the leaf area; lesions may clump together resulting in larger affected areas associated with chlorotic tissues. Some lesions show easily visible gray-colored synnemata (fungal brush-shaped structures).

Grade 7. Abundant large lesions with sporulation, covering nearly 10% of the leaf area. Affected leaves become chlorotic, and the underside of the leaves shows lesion coalescence to form gray-colored areas with high sporulation.

Grade 8. Coalescent lesions with high, gray-colored sporulation on the underside of the leaves, covering between 11% and 24% of the leaf area. Affected leaves become chlorotic, which may or may not cause severe and premature defoliation.

Grade 9. Coalescent lesions with high, gray-colored sporulation on the underside of the leaves, covering 25% or more of the leaf area. Leaflets are chlorotic, which causes severe and premature defoliation.

Generally, growth habits 1 and 2 show earlier defoliation than growth habit 3 under the same degree of infection.

Second evaluation (R6–R7) and third evaluation (R8 middle point)

Grade 1. No disease symptoms in any part of the plant.

Grade 2. Presence of a few small lesions (< 2 mm) with no sporulation. On the pods, such lesions show a reddish-brown color.

Grade 3. Presence of a few small lesions (< 2 mm) with no sporulation, covering approximately 2% of the leaf area, and the pods may show small, not clearly defined, reddish-brown lesions.

Grade 4. Presence of generally small necrotic angular lesions (≥ 3 mm), limited by the primary and secondary veins on the upper side of the leaves, with very scarce sporulation on the underside of the leaves, covering approximately 3% to 4% of the leaf area, and the pods may also show oval or round, slightly concave, reddish-brown lesions.

Grade 5. Presence of several generally medium-sized angular lesions (≥ 4 mm) defined by the veins with limited sporulation on the underside of the leaves, covering approximately 5% of the leaf area. On the pods, reddish lesions are becoming larger and are starting to produce spores and turn gray.

Grade 6. Abundant generally large lesions (≥ 5 mm) on the underside of the leaves, covering between 6% and 9% of the leaf area; these lesions may clump together resulting in larger affected areas associated with chlorotic tissues. On the pods, lesions show well defined, usually round, or sometimes oval, slightly concave edges, and when there is sporulation, they are gray-colored.

Grade 7. Abundant large lesions with sporulation covering nearly 10% of the leaf area. Affected leaves become yellow, and the underside of the leaves shows coalescence of lesions to form gray-colored affected areas with high sporulation. On the pods, lesions show well defined, usually round, or sometimes oval, slightly concave edges, gray-colored when there is sporulation.

Grade 8. Coalescent large lesions with high, gray-colored sporulation on the underside of the leaves affecting between 11% and 24% of the leaf area. Affected leaves become chlorotic, which causes severe and premature

defoliation. The pods show well defined lesions, generally large and gray-colored due to sporulation.

Grade 9. Coalescent lesions with high, gray-colored sporulation on the underside of the leaves, covering 25% or more of the leaf area. Leaflets are chlorotic, which causes severe and premature defoliation. The pods show large lesions with well-defined edges; they are gray-colored due to sporulation.

Generally, growth habits 1 and 2 show earlier defoliation than growth habit 3 under the same degree of infection.

Rating scale for Anthracnose *(Colletotrichum lindemuthianum)* First evaluation: R5

Grade 1. No visible disease symptoms in any part of the plant.

Grade 2. Presence of at least one small (1–2 mm), concave, reddish lesion on the primary vein on the underside of the leaves.

Grade 3. Presence of very few small lesions (1–2 mm), generally on the primary and secondary veins on the underside of the leaves.

Grade 4. Presence of several lesions (3–4 mm) located along the veins; also on petioles, they appear as concave lesions or small spots of a brick red color becoming dark brown over time. The affected area represents between 2% and 4% of leaf the surface; some genotypes show epinasty (downward curving of the leaves), generally of the lower leaves.

Grade 5. Presence of several lesions (3–4 mm) on the petiole, as well as primary and secondary veins, both on the upper- and underside of the leaves. Lesions around the veins show an irregular shape and a reddish-brown color. The affected area represents approximately 5% of the leaf surface. Some genotypes show epinasty of infected leaves.

Grade 6. Presence of numerous large necrotic lesions (≥ 5 mm) on the upper- and underside of the leaves; petioles and stems show chancres with a slight whitish sporulation. The affected area represents approximately between 6% and 9% of leaf the surface.

Grade 7. Presence of numerous large necrotic lesions on the upper- and underside of the leaves. Lesions show an irregular shape and a reddish-brown color around the veins. Petioles and stems also show chancres with a strong whitish sporulation. The affected area represents approximately 10% of leaf the surface; some genotypes show epinasty and leaflets tend to split.

Grade 8. Severe necrosis between 11% and 24% of plant tissue as a result of lesions on the leaves, petioles, stem, and branches. This necrosis often causes the death of part of the plant.

Grade 9. Severe necrosis in over 25% of plant tissue as a result of lesions on the leaves, petioles, stem, and branches. This necrosis often causes plant death.

Second evaluation (R6–R7) and third evaluation (R8 middle point)

Grade 1. No visible disease symptoms in any part of the plant.

Grade 2. Presence of at least one small (1–2 mm), concave lesion generally on the primary vein on the underside of the leaves. The pods show very few small lesions.

Grade 3. Presence of a very few small (1–2 mm), concave, reddish lesions on the primary vein on the underside of the leaves and pods. The affected area represents approximately 1% of the leaf or pod area.

Grade 4. Presence of several lesions (3–4 mm) located along the veins; also, petioles show concave lesions or small spots of a brick-red color becoming dark brown over time. The affected area represents between 2% and 4% of the leaf surface area. Some genotypes show epinasty (downward curving of the leaves), generally of the lower leaves. The pods show round and small lesions (1–2 mm in diameter) with limited or no sporulation.

Grade 5. Presence of several lesions (3–4 mm) on the petiole, as well as on the primary and secondary veins, both on the upper- and underside of the leaves. Lesions around the veins show an irregular shape and a reddish-brown color. The affected area represents approximately 5% of the leaf surface. Some genotypes show epinasty of the infected leaves. The pods show round medium-sized lesions (> 2 mm in diameter) with limited sporulation.

Grade 6. Presence of numerous large necrotic lesions (≥ 5 mm) on the upper- and underside of the leaves; petioles and stems show chancres with a slight whitish sporulation. The affected area represents approximately between 6% and 9% of the leaf surface. The pods show several medium-sized lesions (> 2 mm in diameter), although some small and large lesions may also be visible, generally with whitish sporulation.

Grade 7. Presence of numerous large necrotic lesions on the upper- and underside of the leaves. Lesions show an irregular shape and a reddish-brown color around the veins. Petioles and stems also show chancres with a strong whitish sporulation. The affected area represents approximately 10% of the leaf surface and some genotypes show epinasty and leaflets tend to split. The pods show several medium- to large-sized lesions (2–4 mm in diameter), generally with an abundant whitish sporulation.

Grade 8. Severe necrosis between 11% and 24% of plant tissue as a result of lesions on leaves, petioles, stem, and branches. This necrosis often causes the death of part of the plant. The pods show numerous, large, concave chancres (2–4 mm) with abundant sporulation.

Grade 9. Severe necrosis in over 25% of plant tissue as a result of lesions on the leaves, petioles, stem, branches, pods, and even on the meristem (growing point). This necrosis often causes plant death. The presence of numerous, large, concave chancres with sporulation may cause pod deformation, low seed numbers, and eventually pod death.

Rating scale for common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans)* First evaluation: during R5 stages

Grade 1. No visible disease symptoms in any part of the plant.

Grade 2. Leaf area shows small generally brown-colored and slightly watery spots on the underside of the leaves.

Grade 3. The leaves show small lesions as spots with a wettish aspect on the underside of the leaves, which later increase in size (3–4 mm), and on the upper side of the leaves, lesions appear brown and necrotic, generally growing from the edge towards the center of the leaves.

Grade 4. Less than 5% of the leaf area is covered with irregular lesions (< 6 mm), which generally appear on the edge of the leaf, and may be surrounded by chlorotic halos very visible on the upper side of the leaves. The underside shows slightly watery spots.

Grade 5. Less than 5% of the leaf area is covered by irregularly shaped lesions (≤ 6 mm), which are brown and necrotic on the upper side of the leaves, and darker brown, oily or watery, on the underside. Both the upper- and underside of the leaves show lesions surrounded by a chlorotic halo.

Grade 6. Between 6% and 9% of the leaf area is covered by large lesions (over 6 mm), which show coalescence, are brown, necrotic, watery, and surrounded by chlorotic halos.

Grade 7. Approximately 10% of the leaf area is covered by large lesions (over 6 mm), which are brown, necrotic, and surrounded by chlorotic halos. Some genotypes may show defoliation.

Grade 8. Between 10% and 20% of the leaf area is covered by coalescent, large, generally necrotic lesions of over 6 mm, and plant defoliation starts to occur.

Grade 9. Over 25% of the leaf area is covered by coalescent, necrotic lesions, which clump together, causing plant defoliation.

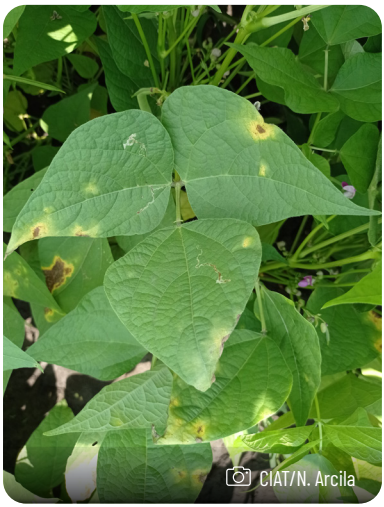
Second evaluation (R6–R7) and third evaluation (R8 middle point)

Grade 1. No visible disease symptoms in any part of the plant.

Grade 2. Leaf area shows small generally brown-colored and slightly watery spots on the underside of the leaves. The pods show small brown and sometimes watery spots.

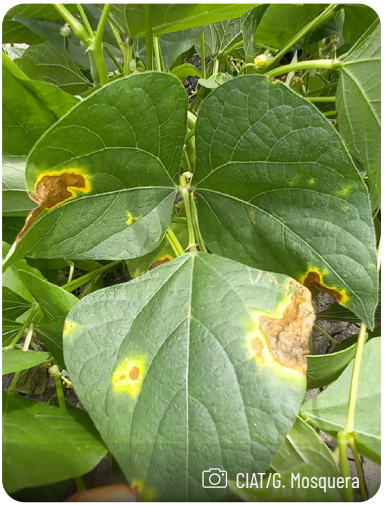
Grade 3. The leaves show small lesions (≥ 3 mm) as spots with a wettish aspect on the underside of the leaves, which later increase in size (3–4 mm), and on the upper side of the leaves, lesions appear brown and necrotic, generally growing from the edge towards the center of the leaves. The pods show a few dark and watery spots.

Grade 4. Less than 5% of the leaf area is covered with irregular lesions (< 6 mm), which generally appear on the edge of the leaf, and may be surrounded by chlorotic halos very visible on the upper side of the leaves. The underside shows slightly watery spots. Lesions on the pods are watery, generally small and do not cover more than 5% of pod surface.



Grade 5. Less than 5% of the leaf area is covered by irregularly shaped lesions (≤ 6 mm), which are brown and necrotic on the upper side of leaves, and darker brown, oily or watery, on the underside. Both the upper- and underside of the leaves show lesions surrounded by a chlorotic halo. Lesions on pods are generally small and sometimes elongated, usually showing a wettish aspect.

Grade 6. Between 6% and 9% of the leaf area is covered by large lesions of over 6 mm, which coalesce on the underside of the leaves, and are brown, necrotic, and watery, surrounded by chlorotic halos. Lesions on pods are medium-sized and sometimes elongated, usually showing bacterial exudates.



Grade 7. Approximately 10% of the leaf area is covered by large lesions (over 6 mm), which are brown, necrotic, and surrounded by chlorotic halos. Some genotypes may show defoliation. Lesions on the pods are large, and they coalesce, and show bacterial exudates.

Grade 8. Leaf area is covered by coalescent, large, generally necrotic lesions and plant defoliation starts to occur. Lesions on pods tend to clump together and show bacterial exudates; pods start to deform.

Grade 9. Over 25% of the leaf area is covered by large, generally necrotic lesions, which clump together, causing plant defoliation. Lesions on the pods coalesce, showing abundant bacterial exudates, which cause pods to deform, and seeds do not develop.

General recommendations for field trials to evaluate bean diseases

A. Establishing nurseries for disease evaluation

Genotypes to be evaluated – early or advanced generations of experimental lines, accessions, or varieties – must be accompanied by reference genotypes. Depending on the generation, seed availability, and area available, it is advisable establish plots with 1–4 rows, 3–5 linear meters long, with no less than 7 plants per linear meter. Additional to the trial, sowing the following could be considered:

1. **Differential varieties:** Include at least two replicates (or more if the area allows – three would be optimal) of differential genotypes for anthracnose (ANT) and angular leaf spot (ALS). Keeping historical records of the differential varieties' behavior allows elucidating races' dynamics over time, i.e., races which persist, races which disappear, or which new races are emerging in the geographical area where the trials are being established.

2. **Checks:** Use resistant, intermediate, and susceptible controls both from the Mesoamerican and Andean genetic pools, across the experimental field. Controls must be sown every 10–20 rows. The infection of the susceptible check across the field provides information on the evenness of pathogen dispersion over the plot. An abundance of checks across the field allows easier comparison of genotypes' behavior under evaluation against controls.

3. **Susceptible checks** for ANT could include CAL96 and MDRK; for ALS, G2858, PAN72, and DICTA17; and for CBB, BAT41 and CAL96. These checks have behaved very consistently in trials conducted in Colombia. Ideally, they should be validated, or new checks should be identified that are susceptible to the pathogen populations in each country.

4. **Spreaders:** A mixture of several susceptible genotypes of both Andean and Mesoamerican origin may be used to increase pathogen dispersion. For natural infection conditions, it is advisable to sow spreaders at least 15 days prior to establishing the nurseries.

B. Inoculum, timeframe for artificial field inoculation, and onset of symptoms.

1. Inoculum concentrations recommended for field trials are:

   ALS: 2 x 10^4 conidia/mL

   ANT: 1.2 x 10^6 conidia/mL

   CBB: 5 x 10^8 UFC

2. Three inoculations must be conducted, one every seven days, starting at the V4 plant growth stage (third trifoliolate leaf), which under a moderate, warm climate, occurs at approximately 21 days after sowing, and under an intermediate climate, at 30 days after sowing. When evaluating genotypes with a long-life cycle, and the last inoculation is made before pods have set, performing a fourth inoculation is advisable.

3. Inoculations should be conducted late in the afternoon when maximum temperatures start dropping and relative humidity increases.

4. The first ALS and CBB symptoms appear between 10 and 12 days after the first inoculation. In the case of ANT, they appear 14 to 20 days after inoculation.

5. At least three evaluations must be carried out in the crop's growth stages R6 (Flowering) and half-way through R8 (mid seed fill).

C. Recommendations for evaluating segregant lines (F3, F4)

To evaluate germplasm showing variation in their responses to pathogens, it is advisable to take two scores of the degree of severity in the following cases:

1. When there are plants with score 1 and plants with any of the other 2–9 scores.

2. When there are plants with degrees of severity differing by more than two scores, for instance, 2 and 4; 4 and 6; 3 and 5; 4 and 7; etc. Such differences in the degree of severity are important because they may be fluctuating between a resistant, intermediate, and susceptible category.

3. When the degree of severity varies between 7 and 9, it may be advisable to record both scores, even though such difference has no practical use, as they fall into the same susceptible category.

4. In addition to the degree of severity, it is advisable to record data on the proportion of diseased plants in relation to the total number of plants (incidence rate – %) corresponding to each degree of severity. Thus, field books should have two columns to record the severity in each evaluation date, and two columns for incidence (Severity A, Incidence A, Severity B, Incidence B). On each evaluation, it is important to record the crop growth stage and age in days after sowing.

Note: It is left to the breeder to decide the fate of materials, according to the severity scores recorded, but it is estimated that if severity scores over 4 are seen in 30% or more plants, the genotype should be discarded, except for those which show another trait of particular interest. Such a consideration is valid for advancing the generation of breeding materials and not for selecting sources of resistance, where the maximum acceptable score is 3.

D. Recommendations for evaluating and selecting disease-resistant parents

The rating scale described may also be used to evaluate germplasm in the field, for selecting parent lines or accessions that confer resistance for breeding purposes.

If a field with good environmental conditions to enable a high and consistent disease pressure (natural inoculum) is not be available for the trial, it is advisable to infect plants artificially e using inoculum prepared in the laboratory. If this is the case, please consider:

1. Inoculate nurseries separately with mixtures of Mesoamerican OR Andean isolates. Select materials showing scores of 1–3. This allows the selection of materials with resistance to one or the other gene pool of isolates.

2. Inoculating nurseries separately with a mixture of Mesoamerican AND Andean isolates. Select materials showing scores of 1–3. This allows the selection of materials showing resistance to the mixture of isolates from both gene pools that may be present in some production areas.

3. Repeating this process at least in two crop cycles to confirm the reaction to pathogens and re-select materials showing scores 1–3.

4. In the case of diseases such as CBB, where it is difficult to find *P. vulgaris* genotypes with high resistance levels, materials showing a score of 4 may be selected.

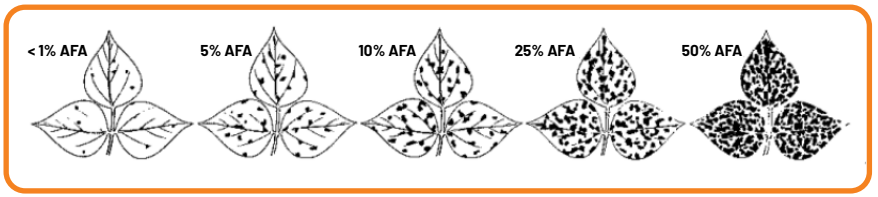
These four recommendations also apply for the selection of parents under greenhouse inoculation conditions, taking into consideration the original rating scale of CIAT's standard system.

E. Additional recommendations

1. Conduct evaluations as early in the day as possible (climate conditions in the morning make the process less tiring).
2. Avoid conducting evaluations at noon (high sunshine intensity may affect visualizing field symptoms).
3. Avoid conducting evaluations under reduced light conditions (when there is not enough sunlight).
4. If possible, ensure the sun is behind your back when evaluating a row.
5. Always check the lower parts of the plants in the row under evaluation. Often, higher disease levels are observed on the lower parts of the plants, and new leaves tend to hide this, resulting in inaccurate plant damage assessments.
6. When conducting the evaluation, walk along the row on both sides.
7. Try not to invest too much time on the evaluation of one row; when in doubt, compare with the reaction of controls.
8. Take a break after conducting evaluations for over two hours and do not conduct evaluations for more than four consecutive hours. Tiredness may affect your judgment.
9. The higher the number of evaluations (three or more), the more trustworthy the assessment. In the case of CBB, it is advisable to place special emphasis on the degree of susceptibility at the mid-seed-filling stage (R8 middle point).
10. For ANT and ALS, damage should be assessed on leaf and pod areas throughout the three evaluations.

Annex I

Scheme for estimating the percentage of leaf area affected by symptoms to be used in the evaluation scale.[1]



1 Modified from Correa, F. 1987. Pathogenic variation, production of toxic metabolites, and isoenzyme analysis in *Phaeoisariopsis griseola* (Scc.) Ferr. PhD Dissertation. Michigan State University.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