

A fluorometric assay to estimate pea leaf weevil (Coleoptera: Curculionidae) larval feeding damage using leghaemoglobin in root nodules of faba bean (Fabaceae)

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Supplementary material: The protocol for legume plant nodulation assessment used at early to late flower stages. The protocol is adapted from 20/20 Seed Lab Inc. 2019.

Assessment Criteria

1. Plant and Growth Vigour

- Plants green and vigorous (healthy) — 5
- Plants green and relatively small — 3
- Plants slightly chlorotic (less green) — 2
- Plants very chlorotic — 1

2. Nodule Colour and Number

- Greater than five clusters of pink pigmented nodules — 5
- Three to five clusters of predominantly pink nodules — 3
- Less than three clusters of nodules, or whitish/greenish nodules — 1
- No nodules, or white/green nodules — 0

3. Nodule Position

- Crown and lateral root nodulation — 3
- Generally crown nodulation — 2

- Generally lateral root nodulation — 1

Total score = score for plant and growth vigour + score for nodule colour and number + score for nodule position

Total Score = 11 to 13

Nodulation assessment: Effective Nodulation

Nitrogen-fixing potential: Good. No further Steps required

Total Score = 7 to 10

Nodulation assessment: Less effective nodulation

Nitrogen-fixing potential: Reduced. Check inoculation method for errors; could also be a result of less-than-optimal growing conditions.

Total Score = 1 to 6

Nodulation assessment: Unsatisfactory

Nitrogen-fixing potential: Poor. Re-evaluate inoculants used, inoculation method and growing conditions.