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OPTIMIZING SPORULATION OF Pseudocercospora griseola IN VITRO

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INTRODUCTION
Angular leaf spot of common bean (ALS), caused by fungus Pseudocercospora griseola (Sacc.) Crous & U. Braun, is one of the major diseases that affect the crop (Singh & Schwartz, 2010). Development of resistant cultivars is one of main objectives of common bean breeding programs and success in selection of lines and progenies depends on accurate assessments of ALS severity. High conidia production in vitro is difficult and makes the use of artificial inoculation in common bean plants unfeasible (Sanglard et al., 2009). Development of efficient methodologies in conidia production to artificial inoculation has been reported in literature, however, average time to obtain enough sporulation is around 30 to 45 days (Sanglard et al., 2009; Silveira 1967). In this study we present a new protocol for sporulation of P. griseola and compare its efficiency with other methods already described in literature.

MATERIAL AND METHODS
Three different protocols were used to sporulation of P. griseola: PDA (potato-dextrose-agar) medium using 200g of potato infusion, 20g dextrose, 20g agar and distilled water to complete 1000 ml; LDA (leaf-dextrose-agar) medium using 200g of common bean leaves, 20g agar, 20g dextrose and distilled water to complete 1000 ml (Silveira 1967); V8 medium using 200 ml of V8 juice, 17g agar, 3g CaCo3 and distilled water to complete 1000ml (Sanglard et al., 2009). Three P. griseola isolates were evaluated: Psg-3 (race 63-63), Psg-5 (race 63-23) and Psg-115. For evaluation in PDA medium each isolate was transferred to test tubes containing approximately 8 ml of medium and evaluation was carried out after 12 days. For LDA and V8 media evaluations, firstly each isolate was transferred to test tubes containing PDA. After 15 days mycelium scraping was performed using 5 ml of distilled water. Subsequently mycelium was transferred to Petri dishes containing LDA or V8 media and evaluation was carried out after 6 days. Petri dishes and test tubes were incubated (BOD) at temperature 24 ° C until evaluation in all methodologies. For obtaining conidia suspension, colony surface was scraped using 5 ml of distilled water and a brush to releasing conidia. Suspension was filtered through gauze and conidia concentration was determined in a Neubauer® chamber. Experiment was conducted in a completely randomized design (CRD) with four replications and nine treatments arranged in a 3x3 factorial, three culture medium (PDA, LDA and V8 medium) and three isolates (Psg-3, Psg-5, Psg-115). Data were submitted to analysis of variance and treatments were compared by the Scott Knott test. Genes® software was used to perform statistical analysis.

RESULTS AND DISCUSSION
Source of variation isolate was statistically significant in analysis variance (Table 1). Number of conidia was 3.9x10⁵ (Psg-3), 4.9x10⁵ (Psg-5) and 9.2x10⁴(Psg-115) conidia.mL⁻¹ for P. griseola isolates evaluated. Psg-3 and Psg-5 isolates formed one group and Psg-115 isolate another one by Scott-Knott's mean test. High rate of sporulation and aggressiveness of Psg-3 isolate (race-63-63) has been reported (Pereira et al., 2015; Pádua et al., 2016). All sporulation protocols evaluated were efficient in production of P. griseola conidia. LDA and V8 media have been successfully
used in conidia production of *P. griseola*, however, time to producing inoculum is long and methodology is laborious (Sanglard et al., 2009; Silveira 1967). Protocol proposed in this work uses PDA medium directly in test tubes to sporulation. However, it is not necessary to transfer mycelium to Petri dishes containing culture medium mentioned above. In this case, time to conidia production from mycelium disc is only 12 days, unlike other protocols that required 30 to 45 days to producing conidia. This is great advantage of this methodology that provides a reduction at least 18 days in production of pathogen inoculum. Furthermore, this method uses only one culture medium, making it faster and more practical. This is the first report of PDA use in test tubes to sporulation of *P. griseola*.

Table 1. Summary of analysis of variance to number of conidia.mL\(^{-1}\) in evaluation of three different protocols to sporulation of three isolates of *P. griseola*.

<table>
<thead>
<tr>
<th>SV</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate (I)</td>
<td>2</td>
<td>83457.69**</td>
</tr>
<tr>
<td>Protocols (P)</td>
<td>2</td>
<td>5154.69</td>
</tr>
<tr>
<td>P X I</td>
<td>3</td>
<td>10172.64</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>98596.50</td>
</tr>
</tbody>
</table>
** Mean\(^1\) 3.2x 10\(^5\)
** Range\(^1\) 2.6 x10\(^4\) to 3.7x10\(^5\)

** Significant at 5% probability, by the F test. SV = source of variation; d.f. = degrees of freedom; MS = medium square; \(^1\) Concentration of conidia /mL\(^{-1}\).

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REFERENCES