Clonal Variation of Eucalypts in Susceptibility to Bacterial Wilt Detected by Using Different Inoculation Methods

By R.-P. Wei1,2,*) , Z. Luo2,3) and B. Fang3)

(Received 23th May 2013)

Summary

Four inoculation methods were investigated for assessing the clonal variation of eucalypts in susceptibility to bacterial wilt (Ralstonia solanacearum). The results showed that these inoculation methods obviously differed in the disease infection process, clonal variation and clonal mean repeatability in susceptibility of stock materials inoculated. For each inoculation method, the clonal effect was consistently significant over the assessment period. Root-collar suspension injection method (RSI) yielded the highest relative clonal variation (0.67 ± 0.086) and clonal mean repeatability (0.92 ± 0.038) in both disease infected incidence and severity at the end of assessment, attributable to the enhanced genetic variation or low environment effect.

For a given inoculation method, an early assessment time might exist for maximizing relative clonal variation or repeatability. It is desirable in breeding to adopt an inoculation method and/or efficient assessment time with high clonal variance component, which would in turn improve the efficiency of clonal screening.

Key words: Eucalypt, Ralstonia solanacearum, bacterial wilt, inoculation, clonal variation, repeatability.

Introduction

Bacterial wilt caused by R. solanacearum is a common bacterium disease on eucalypts and many other crop or wood species (Buddenhagen, 1986; Hayward, 1991; Roux et al., 2001; Old et al., 2003; Pegg et al., 2003; Naidoo et al., 2011). R. solanacearum is soil-borne, and affects eucalypt seedlings or cuttings and young trees by ways of injured roots, stem wounds or through stomata. Bacterial wilt could easily occur and develop under hot and wet conditions during typhoon season each year in the South China (Wu and Liang, 1988; Zhou et al., 2008). Affected trees are often scattered throughout

---

1) Weirptech Bioresources Limited, F, 28/F. Fullview Court, 32 Fortress Hill Road, North Point, Hong Kong.
2) Forest College, South Agricultural University, Wushan, Guangzhou, China.
3) Guangdong Agricultural Academy, Shipai, Guangzhou, China.
*Corresponding author: RUN-PENG WEI. Tel: +852 6229 7361, Fax: +852 2575 7651. E-Mail: runpenwe@163.com


---

24 Silvaes Genetica 63, 1–2 (2014)
stands and show withering, leaf drop, stem death and reduced growth rate (OLD et al., 2003). In few cases, it was also observed that bacterial wilt almost destroyed the whole young stands (LIN et al., 1996; QI, 2002). This disease is recognized as one of the most significant risks for commercial investment in eucalypt plantations in the South China (ZHOUL, 2008).

Variation in susceptibility to R. solanacearum exists among eucalypt species as well as varieties or clones (DIANESE and DRIEST, 1993; OLD et al., 2003; HUANG et al., 2008). This is fundamental for screening and breeding eucalypt clones of resistance. Artificial inoculation is commonly used to test the susceptibility or resistance of host to a pathogen (ATHILAENTIA and EASTBURN, 1997; FANG, 1998; SHI et al., 2000). Different pathogen inoculation methods are expected to yield distinct results of disease infection (DIANESE and DRIEST, 1993; ATHILAENTIA and EASTBURN, 1997; SHI et al., 2000; HÜBNER et al., 2002; RÚZ et al., 2008). However, little effort was made to investigate the likely difference in key genetic parameters in disease infection traits among different inoculation methods, which would affect the effective screening of resistant clones as for other silvicultural traits like growth and wood properties (BORRALHO et al., 1992; ZHANG et al., 2003; ISKR et al., 2005; BALTUNIS and BRAWNER, 2010). In this study, we employed four inoculation methods to test the susceptibility of eucalypt clones to bacterial wilt, and to investigate the clonal or genetic variation under different inoculation methods.

Materials and Methods
Stock species and clones

The stock materials used in the inoculation experiment included the following species or hybrids: Eucalyptus urophylla S.T. Blake, E. grandis W. Hill ex Maiden, E. wettarensis L.D. Pryor, E. camaldulensis Dehnh., E. tereticornis Smith, E. urophylla S.T. Blake x E. grandis W. Hill ex Maiden, E. grandis W. Hill ex Maiden x E. urophylla S.T. Blake, E. grandis W. Hill ex Maiden x E. tereticornis Smith, E. urophylla S.T. Blake x E. camaldulensis Dehnh., and E. saligna Smith x E. exzerta F.V. Muell. These species and/or hybrids have been identified for developing short-rotation (5-7 years) commercial plantations in the South China (QI, 2002; WEI, 2012). Two kinds of clonal materials were used in this study, i.e. well-acclimated and uniform tube (3.1 x 13 cm) cuttings about 4 months old and 25 cm in height, and semi-lignified shoots of 1 year old pot-raised hedge plants. The rooted tube cuttings, qualified for field planting, went through a full nursery culture process of rooting and growing under conditions of controlled rooting hormone, sunlight, temperature, moisture and nutrition, and acclimation or hardening under conditions of natural sunlight, no-fertilizing and minimum water; the semi-lignified shoots of the hedge plants were “standardized” materials for production of rooted cuttings (QI, 2002). As we did not have a balance structure among species and the pedigree information for the hybrids, we treated all species clones as cultivated varieties. In addition, all the clonal varieties included were early exclusively selected for growth purpose. Considering this fact and possibly no phenotypic and genetic correlation between eucalypt growth and bacterial wilt (GAN et al., 2004), we cautiously assumed that these varieties were randomly sampled in terms of bacterial wilt susceptibility or resistance.

Pathogen and inoculum preparation

A single pathogen isolate was used, which in an early study was found most virulent among 11 isolates originating from the main planting regions of eucalypt in the South China (LUO et al., 2013). The purified pathogen isolate with distilled water was maintained at 4°C in refrigerator. Before inoculation, the pathogen was cultured and propagated on a BPY medium (baff extract 3.8 g, peptone 5.0 g, yeast extract 3.0 g, sugar 20 g, agar 15 g, K2HPO4 2.0 g, KH2PO4 0.5 g, MgSO4·H2O 0.25 g, distilled water 1,000 ml, pH = 7.0) at 30°C for 36 hr, and diluted with distilled water as the inoculum (suspension) with a concentration of 3 x 108 cells/ml. This concentration or similarity was found appropriate for inoculation experiment of eucalypt bacterial wilt in some recent studies (DIANESE and DRIEST, 1993; GAN et al., 2004; HUANG et al., 2008; LUO et al., 2013).

Inoculation

Four pathogen inoculation methods were considered in the experiment:

1) Cutting suspension irrigation (CSI): The rooted cuttings with soil were carefully pulled out from the plastic containers (3.1 x 13 cm), replanted in larger plastic bag containers (6 x 15 cm) by filling sterile soil substrate (a mixture of 30% yellow earth and 70% dry peat moss in volume) around (trimmed the dead roots growing out of bottom of the containers before replanting if necessary), and fully irrigated with inoculum suspension in the morning once a day for the first three days in a row. Minor injury might occur to some hair roots during the replanting. The inoculated cuttings were kept moist for the first three days and regularly irrigated with clean water from the fourth day, as required. This method was similar to the conventional pathogen inoculation (FANG, 1998; SHI et al., 2000).

2) Bare-root cutting suspension culture (BSC): The cuttings were carefully pulled out from the plastic tubes, the soil was carefully washed away, the roots were trimmed to 1.0 cm from the taproot axis and 5 cm from the root collar, and the root-trimmed cuttings were cultured in the 100 ml beakers (52 x 72 mm) filled with the inoculum suspension. From the fourth day, clean water was properly added if necessary.

3) Root-collar suspension injection (RSI): Before inoculation, the plastic tube cuttings were fully sprayed with clean water. The inoculum suspension was well injected with a sterile injector at the root-collar of each cutting once a day for the first three days in a row. The inoculated cuttings were regularly irrigated with clean water as required.

4) Shoot suspension culture (SSC): The uniform semilignified shoots with a length of about 30 cm were first collected from the pot-raised hedge plants, and were

25
then trimmed to a length of 22 cm. Leaves at lower part
(about 8 cm length) were removed. The trimmed shoots
were cultured in glass tubes filled with the inoculum
suspension. From the fourth day, clean water was properly
added if necessary. This method or equivalent was
earily adopted for quick testing and screening of resis-
tant or susceptible eucalypt varieties (Wang et al., 2011;
Luo et al., 2013).

One factor (clone) experiment design with multi-replic-
cates was adopted for each inoculation method. In the
experiment, four inoculation methods were separately
applied to test 20 clones each with 6 replicates (cutting
or shoots). The same set of 20 clones were tested with
three inoculation methods (CSI, BSC and RSI), of which
8 clones along with other 12 clones were tested with
SSC. Therefore, there were totally 32 clones included in
the experiment.

The inoculation work was carried out in the morning
of the 9th and the next two days of August 2006. All inoc-
ulated cuttings by using CSI and RSI were placed into
greenhouse with day-time temperature more than 30°C
and relative humidity more than 70%. The inoculated
materials by using BSC and SSC, respectively, were
kept in lab with day-time temperature more than 28°C
(Luo et al., 2013). The inoculated materials were main-
tained under the conditions as described above over a
period by the end of which a stable disease symptom
was reached.

Disease symptom assessment

Disease severity (DS) was rated for each cutting or
shoot on a scale of 0 to 4 (Table 1). Disease development
was assessed on a daily basis for BSC and SSC, but
daily for the first 10 days and at 2-day interval from the
10th day for CSI and RSI. The assessment was completed on the 26th,
11th, 26th and 10th day, respectively, for CSI, BSC, RSI and SSC. Based on the
disease severity scores recorded, the disease incidence
(DI) was obtained by converting all the infected scores to
1 and the healthy scores to 0.

Statistical analysis

By using the GLM procedure of SAS software (SAS
Institute Inc., 1992), ANOVAs were carried out to
examine the clonal effect and variation in susceptibility
traits, i.e. disease severity (DS) and infected incidence
(DI), of eucalypts to bacterial wilt for each inoculation
method under the model:

$$Y_{ij} = \mu + C_i + e_{ij},$$

where $Y_{ij}$ = the observed value, $\mu$ = the mean value of all
clones tested, $C_i$ = the clone effect, $e_{ij}$ = the residue. The
original and transformed values ($\sqrt{Y_{ij}}$) of $D_S$ and $D_I$
were compared in analysis, which turned out the same
or similar conclusions. Therefore, we adopted the
original score or value for both traits throughout the
paper.

For a given trait, the clonal ramet based repeatability
($R_R$), which is identical to the intra-class correlation ($t$),
and its standard error $[Se(R_R)]$ were calculated
using the following equations (Becker, 1992; Baltunis
and Brawner, 2010),

$$R_R = t = \frac{\sigma_C^2}{\sigma_C^2 + \sigma_E^2}, \text{ and}$$

$$Se(R_R) = \frac{(1-t)[1+(s-1)t]}{\sqrt{s(s-1)(n-1)/2}}$$

where $\sigma_C^2$ = the clone variance component, $\sigma_E^2$ = the
within-clone error, $n$ = the clone number, and $s$ = the
number of ramets (i.e. cuttings or shoots) per clone.
The clonal mean based repeatability ($R_C$) and standard error
$[Se(R_C)]$ were estimated as follows (Wright, 1976;
Zhang et al., 2003; Isik et al., 2005; Baltunis and
Brawner, 2010):

$$R_C = \frac{\sigma_C^2}{\sigma_C^2 + \sigma_E^2/s'},$$

$$Se(R_C) = \frac{(1-t)[1+(s-1)t]}{(s-1)/s(n-1)/2}$$

Obviously, $R_R$ and $R_C$, as well as their standard errors,
are closely related to each other. Both parameters could
well represent the clonal or genetic (additive plus non-
additive) variation (Borrelho et al., 1992; Gan et al.,
2004). On the other hand, $R_C$ could directly be used to predict genetic gain from clonal selection (Borrelho et al.,
1992; Zhang et al., 2003; Isik et al., 2005). In addi-
tion, we also calculated the simple correlation among
four inoculation methods by use of the least-squared
means in $D_S$ or $D_I$ of common clones obtained from

<table>
<thead>
<tr>
<th>Class</th>
<th>Score</th>
<th>Criteria of willing (flabby and drooping)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Health and vigorous, no willing symptom</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Leaf tip willing on 2-3 leaves</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Leaf willing on 1/4-1/2 leaves</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>Leaf willing on 1/2-3/4 leaves</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>Leaf willing on more than 3/4-all leaves or dead</td>
</tr>
</tbody>
</table>

Table 1. – Criteria for rating the disease severity of bacterial wilt after inoculation.
**Results and Discussion**

**Wilt symptom development**

Wilting symptom on the infected cuttings or shoots first appeared on the tips of few leaves or shoots and then expanded to the whole (all leaves) cuttings or shoots. As the disease developed, brown and dark discoloration might be observed on the wounds of shoots or roots (Wang et al., 2011). The overall mean infected incidence ($D_I$) and disease severity ($D_S$) were plotted against days after pathogen inoculation for each inoculation method, with the significance tests indicated for the corresponding clone effects (Figure 1). These results well exhibited the disease development process, expected distinction among inoculation methods, and consistent variation among eucalypt clones tested during the disease development (Dianese and Drystig, 1993; Shi et al., 2000; Huberli et al., 2002; Huang et al., 2008; Ruz et al., 2008; Wang et al., 2011). They could provide a good opportunity to examine the clonal variation associated with specific inoculation method.

Leaf or shoot drooping was first observed at day 6, 2, 4 and 1 after inoculation by using CSI, BSC, RSI and SSC, respectively. Subsequently, both $D_I$ and $D_S$ increased rapidly for BSC, RSI and SSC, but slowly for CSI. $D_I$ approximately approached maximum at day 16, 7, 18 and 7, and $D_S$ at day 20, 8, 24, and 9, for CSI, BSC, RSI and SSC, respectively, after pathogen inoculation. Obviously, BSC, RSI and SSC had higher $D_I$ and $D_S$ than CSI that was more or less similar to a real field situation, e.g. wilt infection in nursery or newly planting sites (Lin et al., 1996; Huang et al., 2008; Luo et al., 2013). In terms of the infection process norm and period, BSC and SSC were similar while RSI was relatively close to CSI. At the end of assessment, RSI resulted in the highest $D_I$ and $D_S$ (0.60 and 2.4), followed in order

---

**Figure 1.** – Average disease infected incidence ($D_I$) and severity ($D_S$) against days after inoculation for four inoculation methods. The letters associated with the average values indicated the significance level of the test of the difference among clones: –, not available; a, $P > 0.05$; b, $0.01 < P \leq 0.05$; c, $0.001 < P \leq 0.01$; d, $P \leq 0.0001$. 

---

27
Clonal variation

Except for no or low infection at the initial stage, the clones tested significantly differed in $D_I$ and $D_S$ no matter which inoculation method was used (Figure 1; Table 3). The result indicated a high degree of variation among eucalypt clones and/or species tested (Dianese and Destigt, 1993; Old et al., 2003; Gan et al., 2004; Huang et al., 2008). In general, the significance level of the test of the difference in $D_I$ and $D_S$ steadily increased with days after inoculation when using CSI and RSI, but showed a confusing pattern under BSC and SSC. By using BSC, the clones differed in both $D_I$ and $D_S$ with $P<0.001$ at day 2 and $P<0.0001$ at day 3, and then gradually downgraded to $P=0.012$. By using SSC, the clones differed in $D_I$ and $D_S$ with $P<0.0001$ for the first 5 and 7 days respectively, and with $P<0.01$, much weak but still significant, for the rest of assessment.

Clonal ramet based repeatability ($R_{R}$) or intra-class correlation ($t$) measured the fraction of the clonal or genetic variation in the total phenotypic variation (Becker, 1992; Baltunes and Branner, 2010), which was presented against days after inoculation for each inoculation method in Figure 2. $R_{R}$ in $D_I$ and $D_S$ for CSI and RSI were initially low, rapidly increased within 10 to 14 days, and subsequently reached a stable value till the end of assessment. Changing in another pattern, $R_{R}$ in $D_I$ and $D_S$ for BSC and SSC increased from a relatively high value at the beginning, reached a peak the next day, and then quickly dropped to values significantly lower than those for CSI and RSI. Difference between BSC and SSC was small. The changing pattern of inoculation methods might imply the complicated role in the infection process of the external conditions including the imposed physiological status (in-vitro culture, tissue injury, vigour etc.) of materials, culture environment and their interaction, besides the genetic aspect of materials.

At the end of assessment, $R_{R}$ in both $D_I$ and $D_S$ converged at the same value for all inoculation methods because the cuttings or shoots inoculated either survived or died. RSI yielded the highest $R_{R}$ (0.67 ± 0.085), followed in order by CSI (0.45 ± 0.106), SSC (0.22 ± 0.096) and BSC (0.15 ± 0.088). This ranking was not fully in consistent with that based on $D_I$ or $D_S$ values (Figure 1). It seemed that RSI had relatively lower standard error of $R_{R}$ as well as $R_{C}$ (Table 2) than other inoculation methods. For each inoculation method, a specific early day appeared with the highest $R_{R}$ in both $D_I$ and $D_S$ (Figure 2), which probably better revealed genetic or clonal variation during the disease development. In other word, the genetic or clonal effect might be relatively enhanced or the environment effect lowered at a specific time.

Clonal mean based repeatability

Closely related to the intra-class correlation or clonal ramet based repeatability, the clonal mean based repeatability ($R_{C}$) varied in a similar pattern but with higher value over time as indicated in Figure 2. In general, $R_{C}$, ranged from moderate for BSC and SSC to high for CSI and RSI. At the final assessment of four inoculation methods, RSI yielded the highest $R_{C}$, followed in order by CSI, SSC and BSC (Table 2). In the phenotypic expression of clonal materials, the genetic variance component includes additive and non-additive effects (Borrallho et al., 1992; Zhang et al., 2003; Isik et al., 2005). As both additive and non-additive gene actions were suggested important for bacterial wilt resistance (Neto et al., 2002; Gan et al., 2004), the enhanced clonal variation or high clonal mean repeatability could be efficiently utilized to select and deploy resistant clones in practice.

If an early assessment was preferred, CSI and RSI were found roughly at day 18 and 12, respectively, to have high $R_{C}$ that was the same as or similar to that at the final assessment (referred to Figure 2). In contrast, BSC and SSC yielded maximum $R_{C}$ in $D_I$ and $D_S$ at day

<table>
<thead>
<tr>
<th>Inoc method</th>
<th>Early assessment with maximum $R_C$</th>
<th>Final assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$D_{I}$ Mean</td>
<td>$R_C$ Mean</td>
</tr>
<tr>
<td>CSI</td>
<td>0.18 ± 0.035</td>
<td>0.70 ± 0.139</td>
</tr>
<tr>
<td>BSC</td>
<td>0.21 ± 0.037</td>
<td>0.43 ± 0.085</td>
</tr>
<tr>
<td>RSI</td>
<td>0.60 ± 0.045</td>
<td>2.15 ± 0.170</td>
</tr>
<tr>
<td>SSC</td>
<td>0.22 ± 0.038</td>
<td>0.85 ± 0.129</td>
</tr>
</tbody>
</table>

by SSC (0.54 and 2.2), BSC (0.35 and 1.4) and CSI (0.18 and 0.7) (Figure 1; Table 2).

**Table 2.** Mean value, clonal mean repeatability and their standard errors (in parenthesis) of the disease infected incidence and severity at final and early assessment with maximum $R_C$ for four inoculation methods.
2 or 3, which were however much higher than those at the final assessment, greatly narrowing their difference with other two inoculation methods (Table 2). Therefore, an efficient inoculation method should simultaneously accelerate the infection process of disease (Figure 1) and favour a better and earlier expression of genetic effect (Figure 2). The high clonal mean repeatability was achieved through enhanced genetic variance or low environment effect (ERICSSON, 1997).

Although RSI was superior in detecting the disease infection of bacterial wilt and the expression of genetic effect, it seemed that the level of disease infection (Di and Ds) was not clearly related to the relative genetic variation (RC). In comparison to other inoculation methods, CSI had a much low Di and Ds but still high RC in both traits (Tables 2 and 3; Figures 1 and 2). Both BSC and SSC had significantly lower Di and Ds but maximum and much higher RC at day 2 or 3 than at the final assessment (day 10 and 11). The superiority of RSI might be attributed to the fine control of the environment effect (ERICSSON, 1997) during the puncturing inoculation.

![Table 3](image-url)

Table 3. – Clone means, overall mean and their standard errors (in parenthesis) of the disease incidence (Di) and severity (Ds) of infected bacterial wilt at final assessment for four inoculation methods.

<table>
<thead>
<tr>
<th>Clone</th>
<th>CSI</th>
<th>BSC</th>
<th>RSI</th>
<th>SSC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Di</td>
<td>DS</td>
<td>Di</td>
<td>DS</td>
</tr>
<tr>
<td>C01</td>
<td>0.83(0.167)</td>
<td>3.33(0.667)</td>
<td>0.50(0.224)</td>
<td>2.00(0.894)</td>
</tr>
<tr>
<td>C02</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.33(0.211)</td>
<td>1.33(0.843)</td>
</tr>
<tr>
<td>C03</td>
<td>0.17(0.167)</td>
<td>0.67(0.667)</td>
<td>0.50(0.224)</td>
<td>2.00(0.894)</td>
</tr>
<tr>
<td>C04</td>
<td>0.17(0.167)</td>
<td>0.67(0.667)</td>
<td>0.33(0.211)</td>
<td>1.33(0.843)</td>
</tr>
<tr>
<td>C05</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.67(0.211)</td>
<td>2.67(0.843)</td>
</tr>
<tr>
<td>C06</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.67(0.211)</td>
<td>2.67(0.843)</td>
</tr>
<tr>
<td>C07</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.33(0.211)</td>
<td>1.33(0.843)</td>
</tr>
<tr>
<td>C08</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
</tr>
<tr>
<td>C09</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.33(0.211)</td>
<td>1.33(0.843)</td>
</tr>
<tr>
<td>C10</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
</tr>
<tr>
<td>C11</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
</tr>
<tr>
<td>C12</td>
<td>0.33(0.211)</td>
<td>1.33(0.843)</td>
<td>0.33(0.211)</td>
<td>1.33(0.843)</td>
</tr>
<tr>
<td>C13</td>
<td>0.33(0.211)</td>
<td>1.33(0.843)</td>
<td>0.50(0.224)</td>
<td>2.00(0.894)</td>
</tr>
<tr>
<td>C14</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.17(0.167)</td>
<td>0.67(0.667)</td>
</tr>
<tr>
<td>C15</td>
<td>0.67(0.211)</td>
<td>2.67(0.843)</td>
<td>0.67(0.211)</td>
<td>2.67(0.843)</td>
</tr>
<tr>
<td>C16</td>
<td>0.83(0.167)</td>
<td>3.33(0.667)</td>
<td>0.83(0.167)</td>
<td>3.33(0.667)</td>
</tr>
<tr>
<td>C17</td>
<td>0.17(0.167)</td>
<td>0.67(0.667)</td>
<td>0.50(0.224)</td>
<td>2.00(0.894)</td>
</tr>
<tr>
<td>C18</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.33(0.211)</td>
<td>1.33(0.843)</td>
</tr>
<tr>
<td>C19</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
</tr>
<tr>
<td>C20</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
<td>0.00(0.000)</td>
</tr>
<tr>
<td>C21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C26</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C29</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean: 0.18(0.035) 0.70(0.139) 0.35(0.044) 1.40(0.175) 0.60(0.045) 2.40(0.180) 0.54(0.046) 2.17(0.183)
Correlation between inoculation methods

Simple correlation between two inoculation methods was essentially the same or equivalent for both disease infection traits (D_I and D_S) at the final assessment. All infected cuttings or shoots finally had either 0 or 1 for D_I, and correspondingly 0 or 4 for D_S. Based on the least-squared means in D_I or D_S of common clones included, the pair correlation was calculated, which was similar to the genetically expressed interaction in the disease infection traits between two inoculation methods or Type-B genetic correlation (BURDON, 1977; JOHNSON, 1997; ZHANG et al., 2003; BALTONIS and BRAWNER, 2010).

Except for between CSI and SSC, all inoculation methods were significantly positively correlated to each other (Table 4). The exception without significant correlation was probably caused by the large error associated with the small sample size, i.e. 8 common clones shared by CSI and SSC. BSC had strong relationship with RSI (P < 0.0001) and CSI (P = 0.006), while other correlations were significant at level of P < 0.05. SSC had significant correlation with BSC and RSI at level of P < 0.05, probably attributable to the relatively similar physiological status of materials inoculated. Similar relationship among four inoculation methods could be found when the maximum R_C at the early assessment was considered. In contrast to the difference in the disease infection process and clonal variation described above among inoculation methods (Figures 1 and 2; Tables 2 and 3), the correlation analysis confirmed the similarity, effectiveness and relative efficiency of four inoculation methods in testing the susceptibility or resistance of eucalypts to bacterial wilt, revealing the genotypic expression, and screening potentially resistant clones (SHI et al., 2000; HUANG et al., 2008).

Conclusions

Four inoculation methods investigated highly differed in the disease infection process, incidence and severity,
and the corresponding clonal variation or clonal mean repeatability. The difference in detecting the clonal variation among inoculation methods were reduced when an efficient early assessment was considered. The difference also indicated the possibility of developing efficient inoculation method with relatively enhanced genetic variation or low environment effect in eucalypt bacterial wilt. This conclusion may be generalized to all quantitative traits in genetic testing where reduced environment effect is expected to relatively increase the genetic variation or the accuracy of breeding value prediction. Four inoculation methods were also significantly correlated, implying that they were all effective in testing the susceptibility or resistance of eucalypts to bacterial wilt, and revealing the genotypic expression of the relevant traits.

Among four inoculation methods, the relative clonal variation in susceptibility of eucalypts to bacterial wilt varied but was consistently significant over the whole infection process of pathogen inoculated. An efficient early assessment could be found to obtain high or maximum clonal variation or clonal mean repeatability. The enhanced clonal variation would benefit the improvement of eucalypts resistance to bacterial wilt. With relatively uniform or controllable inoculation operation, root-collar suspension injection (RSI) yielded the highest clonal variation or clonal mean repeatability with less biased estimates (low standard errors), overwhelmingly superior to other three inoculation methods tested. Nevertheless, further investigation is required to explore other efficient inoculation methods, and to improve the efficiency of existing efficient methods from other aspects such as host materials, pathogen inoculum, environmental conditions, etc.

References


Experimental strategies for clonal eucalyptus

By M. H. S. Mendes¹†, L. N. Rosse², F. H. R. B. Toledo³ and M. A. P. Ramalho⁴

(Received 23rd May 2013)

Abstract

The success of any forestry operation depends on the careful choice of the clones to be planted. At existing yield levels, the differences among clones are becoming smaller, leading to the need to use experimental strategies for a more successful choice. To obtain information in regard to better experimental strategies, data from two clonal tests of eucalyptus were used. One of the tests consisted of 35 clones and another of 48 clones. In both tests, the experimental plot consisted of 100 plants at commercial spacing (12 m² per plant). The trait wood volume was evaluated, taking the relative position of the plant in the plot as a reference. The data were evaluated by different means, and it was observed that the use of border plot in clonal tests in eucalypt clones is not necessary. Experimental accuracy with 15 to 20 plants is similar in comparison to the plot with 100 plants.

Key words: Eucalyptus, experimental precision, accuracy, plot size.

Introduction

In a eucalyptus genetic breeding program, the clonal evaluation stage, especially that which precedes planting recommendation on a commercial scale, is one of the most expensive because it requires that the experiments be set up in representative environments of the operational area of the company. The clonal tests are used for this purpose because they simulate the conditions to which the clone will be subjected on a small scale. For selection to be successful in this phase of the program, evaluating the greatest number of clones possible is indispensable and the test plots must be highly representative. When all this information is analyzed together, it may be clearly perceived how difficult and expensive operationalization of a breeding program is. Therefore, alternatives to facilitate and decrease the cost of this phase of the program should be considered, and one of them is in regard to carefully defining the size of the plots and/or number of plants to be evaluated in the final stages of the program.

Theoretically, the larger the plot, the greater the accuracy. However, the cost of each plot increases with an increase in the number of plants. Thus, identifying this size so as to have efficiency in the recommendation of new clones at the lowest possible cost is fundamental. Normally, to obtain information in regard to the form and the size of the experimental plots, an experimental area is planted to a single clone such that at the time of data collection, the area is split into basic units of minimum size (Paranaiba et al., 2009; Silva, 2010). This pro-