Somaclonal Variation as a Breeding Tool in American Chestnut

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Outline

• Introduction:
  • Disease susceptibility in American chestnut
  • Somatic embryogenesis
  • Somaclonal variation
  • Somatic embryogenesis in *C. dentata*

• Objectives and Hypotheses

• Methodology:
  • Indirect somatic embryogenesis
  • Testing for disease tolerance
**Disease Susceptibility**

- Chestnut blight
  - *Cryphonectria parasitica*
- Ink disease
  - *Phytophthora* sp.
  - Principally *P. cambivora* and *P. cinnamomi*
- *C. dentata* is highly susceptible to both diseases.
  - Asian *Castanea* spp. are tolerant
  - Disease tolerance in *C. dentata* pursued by backcross breeding to Asian *Castanea* spp.

(Burnham 1988; Jeffers 2009)
Somatic Embryogenesis

• Small explants of plant tissues → meristematic tissues → embryos → complete plantlets.
• “Somatic plantlets”

(www.eplantscience.com)
Somaclonal Variation

- Product of somatic embryogenesis.
- Regenerated plantlets have a wide range of phenotypic variations.
  - Known as somaclonal variants.
  - Includes chromosome alterations, epigenetic changes, one time deletions or insertions, transposable elements, etc.
- Disease tolerance is commonly affected in somaclonal variants.
- It’s a numbers game:
  - Undirected changes

(Scowcroft 1985; Wang and Wang 2012)
Somatic Embryogenesis: 
*C. dentata*

- Current somatic embryogenesis:
  - Andrade and Merkle 2005

- Direct somatic embryogenesis:
  - Explant = tissues destined to become embryos

- Explant:
  - Immature zygotic embryos

- Limitations:
  - Explants can only be harvested for 2 weeks midsummer
  - Low explant induction rates (5-6%)
  - Zygotic embryos = heterozygotes of unknown phenotype
Somatic Embryogenesis: *C. dentata*

- Indirect somatic embryogenesis:
  - Somatic embryogenesis from vegetative tissues
    - Leaves, stems, etc.
  - Possible with *C. sativa*
  - Never investigated with *C. dentata*

- Benefits:
  - Year-round somatic embryogenesis for *C. dentata*
  - Possible higher induction rates (29% in *C. sativa*)
  - Vegetative tissues = possible to use mature trees as explants
    - Somatic embryogenesis with elite trees

(Ballester et al. 2001; Corredoira et al. 2005)
Objectives and Hypotheses

• Objectives:
  • To develop a methodology for indirect somatic embryogenesis of *C. dentata*.
  • To increase through somaclonal variation, the disease tolerance of *C. dentata* to chestnut blight and ink disease.

• Hypotheses:
  • Ideal explants will be identified for indirect somatic embryogenesis in *C. dentata*.
  • Somaclonal variants with increased disease tolerance will be generated.
Indirect Somatic Embryogenesis

• Seedlings are extremely vigorous
  • Make excellent explants for woody species

• Explant types tested in this study:
  • Cotyledon
  • Epicotyl
  • Hypocotyl
  • True root tips
  • True leaves

• Two media procedures tested:
  • Andrade and Merkle (2005) procedure for zygotic embryos
  • Ballester et al. (2001) procedure for leaf explants for C. sativa
Determining Disease Tolerance

• New leaf assay technique from Newhouse et al. (2014).
• Relative measure of disease tolerance
• Each somatic line compared to:
  • Positive control: resistant *C. mollissima*
  • Negative control: known susceptible *C. dentata*
  • Somatic variation control: source plant of the somatic line
(Newhouse et al. 2014)
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Questions?
Literature Cited


• Burnham CR (1988) The restoration of the American chestnut: Mendelian genetics may solve a problem that has resisted other approaches. Am Sci 76:478-487


