# Microsatellite-based Analysis of Tobacco Genetic Resources*

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* Moon *et al.* (2009) Crop Sci. 49: 2149-2159.

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~**4,000** *N. tabacum* cultivars, breeding lines, and other primary genetic resources

# Importance of Genetic Resources

- Environmental change, new pest emergence
- Potential for changes in USDA regulation and the development of new market types

Table 3. Average genetic similarity values ($S_{ij}$) for tobacco cultivars of eight different periods.

| Time period | No. of cultivars | Average $S_{ij}$ | Average $S_{ij}$ SE |
|---|---|---|---|
| Initial gene pool | 37 | 0.758 | 0.023 |
| 1940s | 16 | 0.694 | 0.020 |
| 1950s | 11 | 0.704 | 0.030 |
| 1960s | 11 | 0.796 | 0.021 |
| 1970s | 10 | 0.763 | 0.027 |
| 1980s | 11 | 0.776 | 0.016 |
| 1990s | 10 | 0.849 | 0.032 |
| 2000s | 11 | 0.874 | 0.012 |

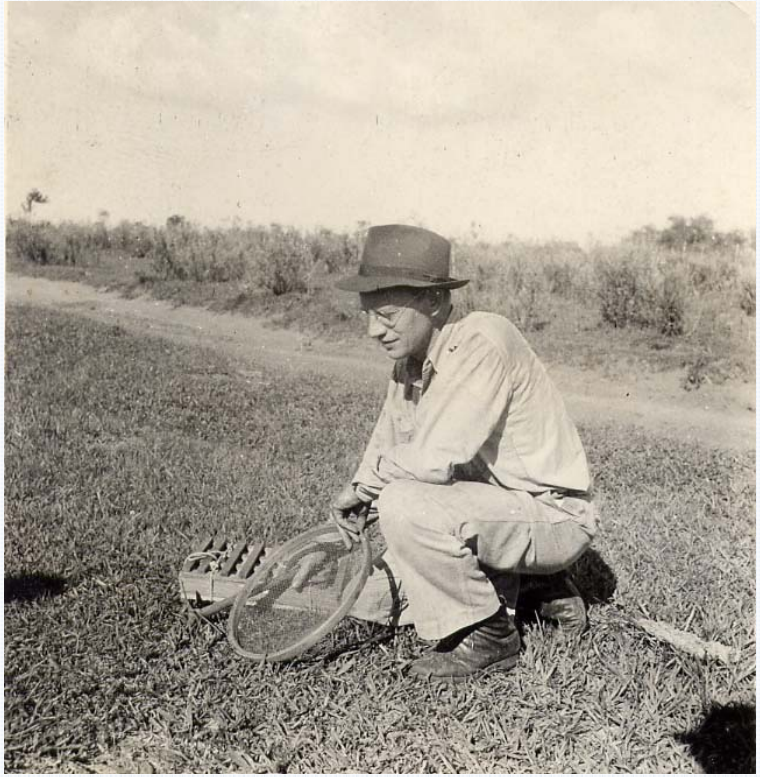
Crop Sci. 49:498–508 (2009).

# Utility of Germplasm Collections

- **TI 448**, mixed sample collected in Colombia in 1936 (Stadelman); source of bacterial wilt resistance
- **TI 706**, 'Copanico Flor Rojo' from Honduras has tolerance to infection by root-knot nematodes; '**Ambalema**' & two dozen other accessions with symptomless resistance to *Tobacco Mosaic Virus* (TMV)
- Other TIs possess notable levels of resistance/ tolerance to black root rot, brown spot, and fusarium wilt
- Disease resistance obtained through interspecific hybridization
  - TMV (*N. glutinosa*)
  - Black shank (*N. plumbaginifolia*)
  - Black root rot (*N. debneyi*)
  - Wildfire (*N. rustica*)

# U.S. *Nicotiana* Germplasm Collection

- Approximately 2,200 accessions, of which ~2,000 are *N. tabacum*
- Accounts for half of the total germplasm available through the CORESTA cooperative
- Only collection that contains a wide array of "primitive" tobacco accessions collected from South and Central America



W. Andrew Archer, 1936

# Adaptability of Tobacco

- Arid highlands of Peru & Ecuador (3,000+ m)
- Greater Orinoco River Basin (Colombia, Venezuela)
- Costa Rica, Honduras & Guatemala
- Northeastern China
- Indonesia & the Philippines
- Malawi & Uganda




# Microsatellite-based Analysis of Tobacco (*Nicotiana tabacum* L.) Genetic Resources

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- **702 TIs** entered into the U.S. Collection prior to 1938
  - 35 countries
  - 88% from Central & South America
- **70 SSR markers** from 23 linkage groups (Bindler et al., 2007)

# Comparison between TI collection and flue-cured germplasm

| Population | No. samples | No. markers | Total no. alleles | Average no. alleles per marker | Average gene diversity |
|---|---|---|---|---|---|
| FC cultivars* | 117 | 70 | 293 | 4.19 | 0.2486 |
| TI collection | 702 | 70 | 1031 | 14.7 | 0.7362 |

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# Genetic diversity measures of TIs collected from 10 Central and South American countries

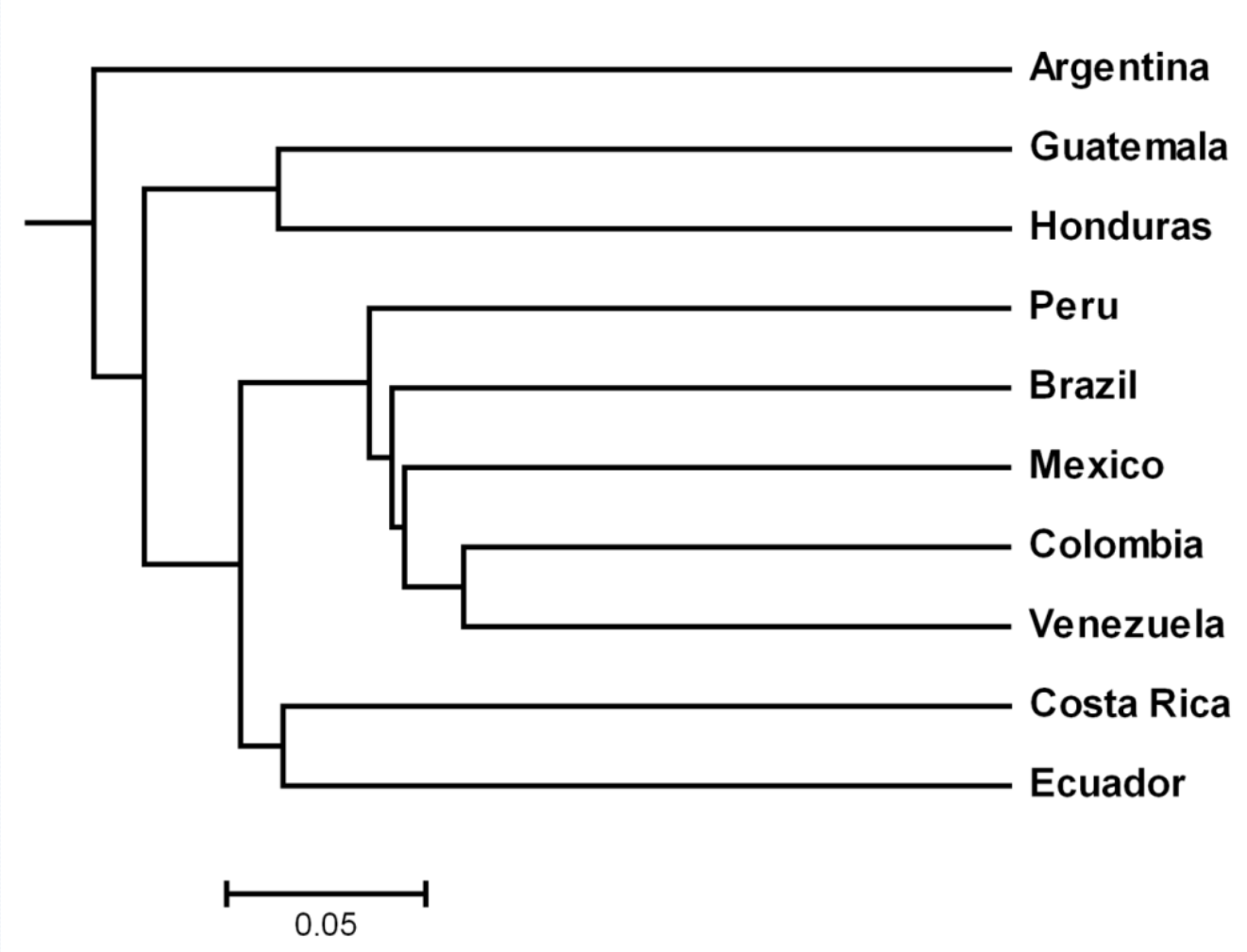
| Country specific subpopulation | No. of samples | No. of alleles | No. of unique alleles | Allelic richness | Average gene diversity | Average genetic distance* |
|---|---|---|---|---|---|---|
| Honduras | 24 (0)† | 383 | 3 | 4.538 | 0.5682 | 0.5297 |
| Argentina | 25 (0) | 422 | 8 | 4.942 | 0.6287 | 0.5837 |
| Guatemala | 25 (1) | 471 | 16 | 5.437 | 0.6736 | 0.6270 |
| Ecuador | 28 (0) | 388 | 3 | 4.461 | 0.5867 | 0.5507 |
| Peru | 33 (4) | 514 | 13 | 5.712 | 0.7143 | 0.6600 |
| Brazil | 34 (4) | 510 | 0 | 5.559 | 0.7011 | 0.6460 |
| Costa Rica | 42 (2) | 546 | 17 | 5.564 | 0.6932 | 0.6339 |
| Colombia | 71 (2) | 597 | 5 | 5.316 | 0.6589 | 0.6004 |
| Venezuela | 136 (6) | 670 | 20 | 5.191 | 0.6375 | 0.5775 |
| Mexico | 187 (30) | 784 | 48 | 5.819 | 0.7049 | 0.6316 |
| Totals | 605 (49) | 1009‡ | 133 | 6.277§ | 0.7278¶ | 0.6522# |

* Chord distance (Cavalli-Sforza and Edwards, 1967)
† Number of probable contemporary U.S. cultivars included in parentheses.
‡ Total number of unique alleles within this population of 605 TIs.
§ Standardized (rarefaction) estimate of allelic richness for 605 TIs.
¶ Overall average gene diversity within this population of 605 TIs.
# Average genetic distance between any two individuals in this analysis.

# AMOVA performed on ten subpopulations collected from Central and South America

| Source of variation | Degrees of freedom | Sum of squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Between subpopulations | 9 | 2416.722 | 4.0047 | 7.82*** |
| Within subpopulations | 595 | 28094.809 | 47.21817 | 92.18*** |
| **Total** | **604** | **30511.531** | **51.22286** | **-** |

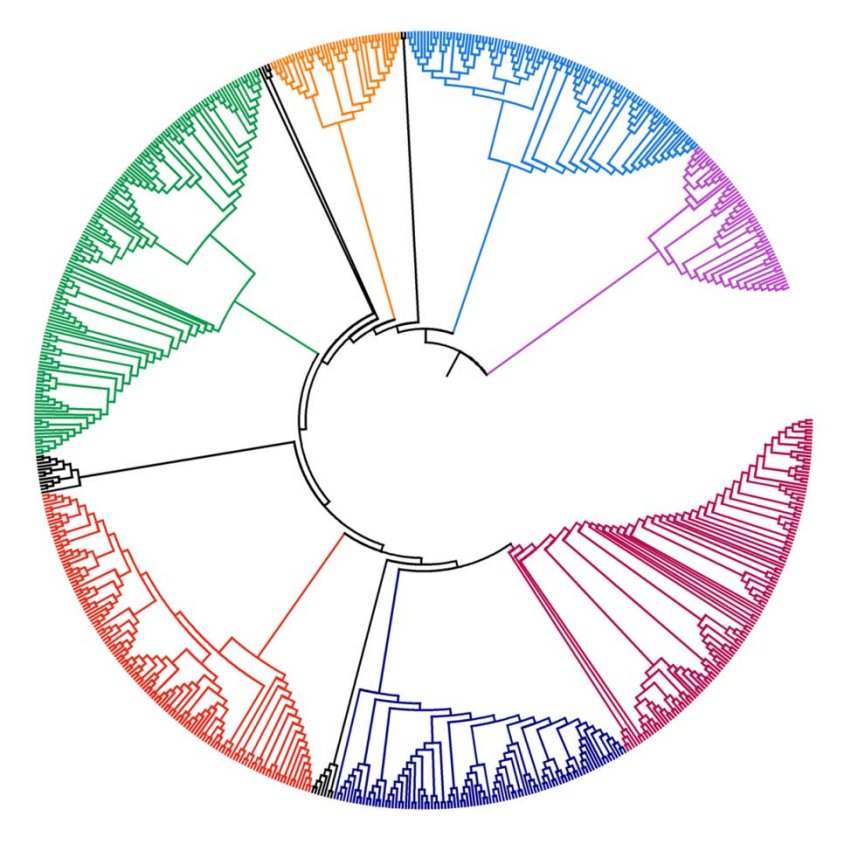
# UPGMA Clustering



# Complete Phylogeny

**Seven consistent clades**

- Mexico
- Oriental
- Argentina/Brazil
- Central America
- Northern S. America, Orinoco River Valley
- Mexico & U.S. materials



# Acknowledgements

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