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Transgenic Loblolly Pine and Importance of Somatic Embryogenesis Scale-up Technologies for Future Commercialization





Loblolly Pine – Most Economically Valuable Tree in the World

- Global pine plantations are expansive and represent a significant source of fiber worldwide
- Loblolly pine in Southeastern U.S.
  - Dominant tree species on 29 million acres[5]
  - 50% of standing pine volume[5]
  - >800 million trees planted annually in Southeastern U.S. (81% of conifer production)[6]
  - Primary markets: pulp, paper, lumber
  - Upside with emerging biomass markets
  - Loblolly pine presents a significant economic opportunity as a tree crop on marginal lands

Pine Planted Area in Major Markets

	Total Planted Area	
	MM hectares	MM acres
Southeastern U.S.[1]	13.4	33.0
Brazil[2]	2.0	4.9
New Zealand[3]	1.6	4.0
Chile[2]	1.5	3.7
Australia[4]	1.0	2.5
Argentina[2]	0.5	1.2
Venezuela[2]	0.5	1.2
Uruguay[2]	0.1	0.3
Colombia[2]	0.1	0.2
TOTAL	**20.7**	**51.1**

[1]Southern Forest Resource Assessment, 2007

[2]BRACELPA, ABRAF Annual Report, 2007

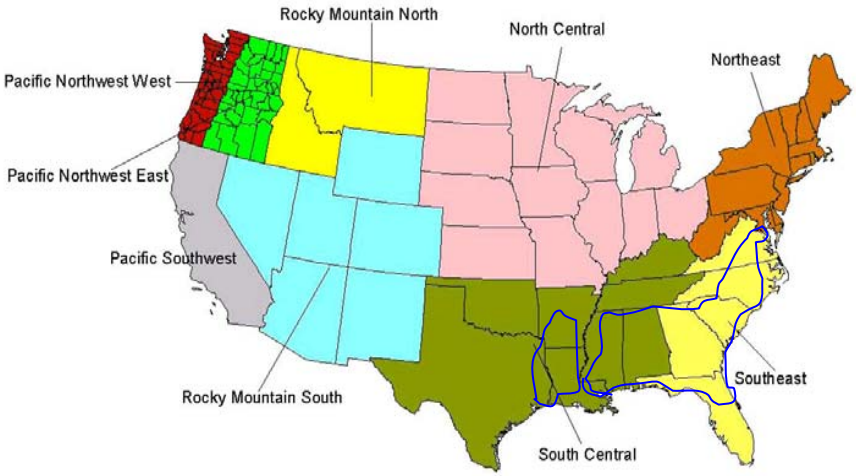
[3]New Zealand National Exotic Forest Description, 2007

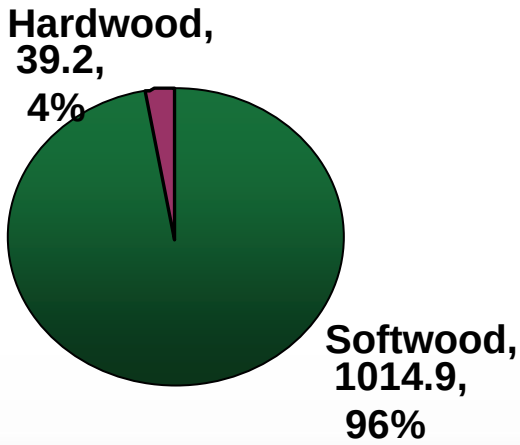
[4]Australian Forest and Wood Products Statistics – March and June Quarters 2008, Australian Bureau of Agricultural and Resource Economics

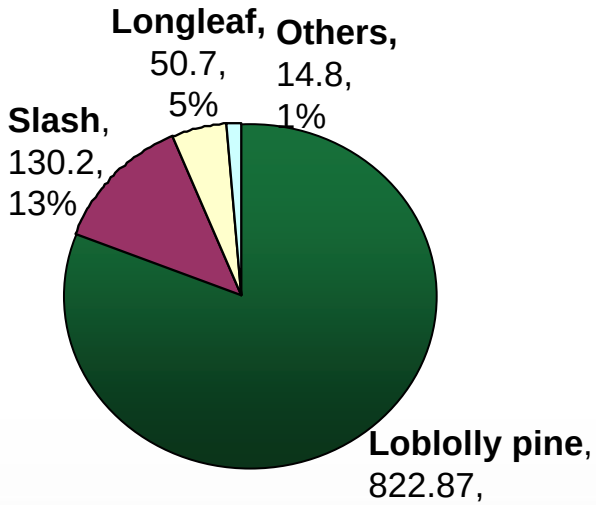
[5]Nix, S. Loblolly Pine: An Important Tree in North America

[6]Auburn University Southern Forest Management Cooperative

Distribution of Loblolly Pine







800 MM seedlings planted annually

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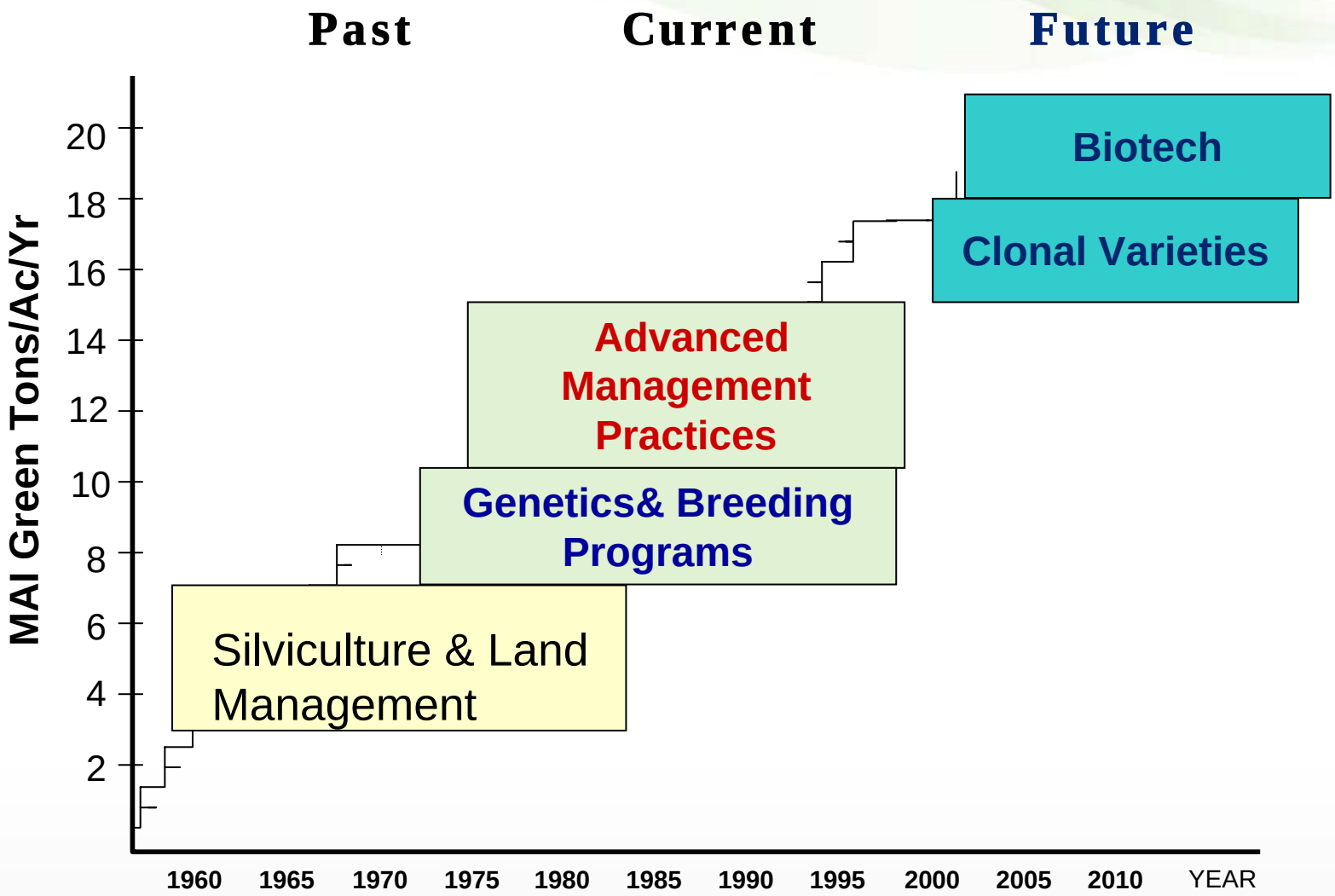
Varietals and biotech offer great potential to accelerate the improvement and value of loblolly pine

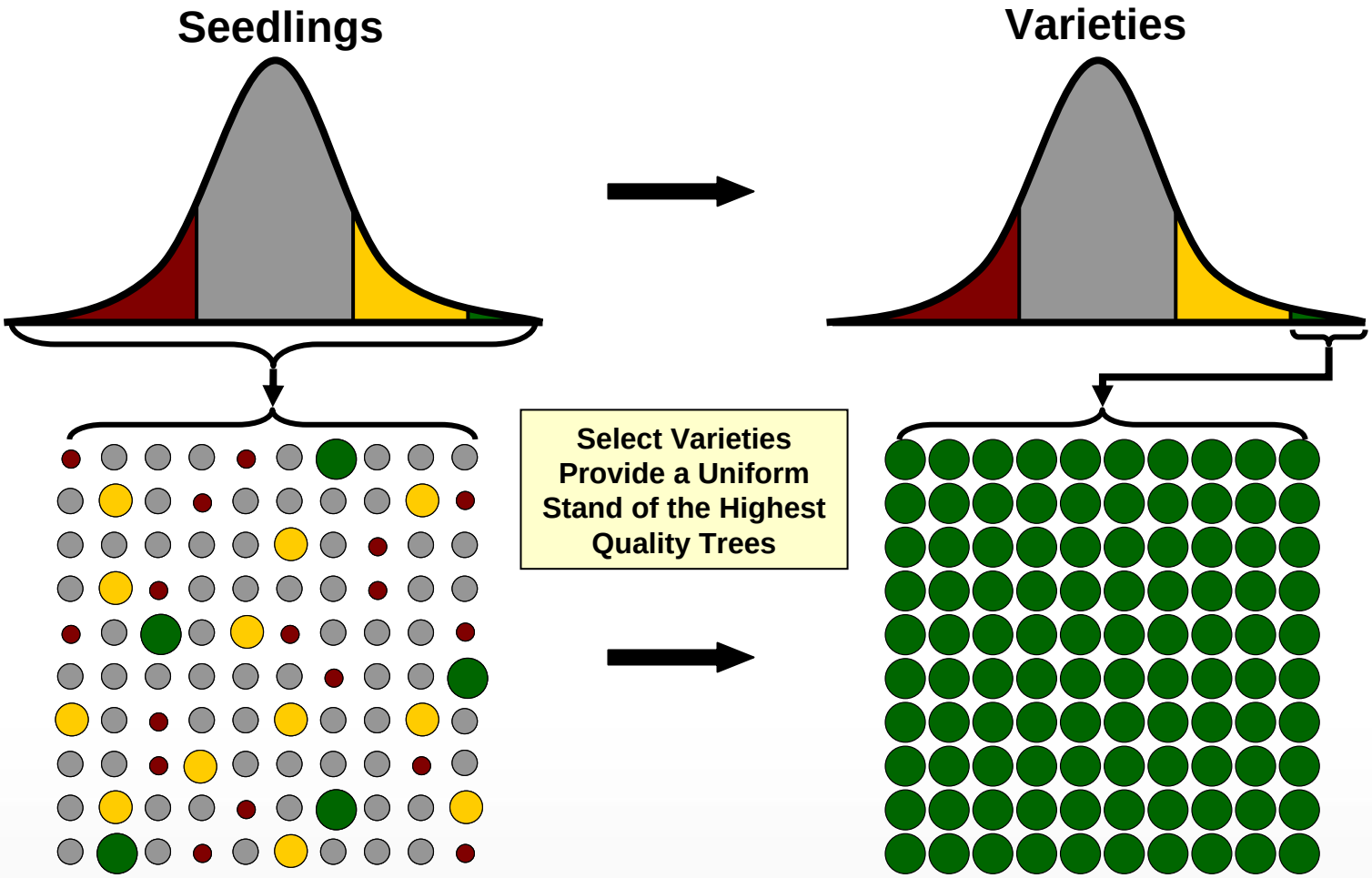




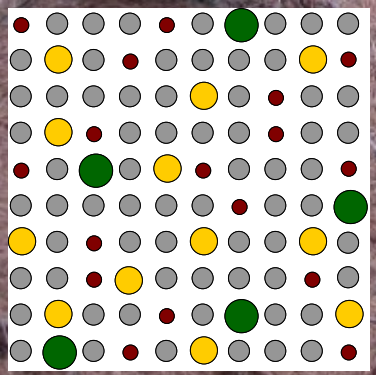
Technology Ladder Toward Improved Productivity



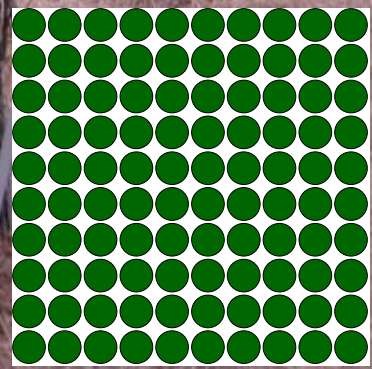
Varietal Technology Allows ArborGen to Select the Highest Performing Varieties



Improvements with Varietals



- Improved Consistency
- Improved Quality
- Faster Growth



Biotech Improves the Best Varieties

- Growth Traits Demonstrated in Pine
- 100% volume gain
- Projected 20 yr vs. 25 year rotation

- Pollen Control Demonstrated

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Production and deployment of biotech Loblolly Pine depends on SE-based varietal technology

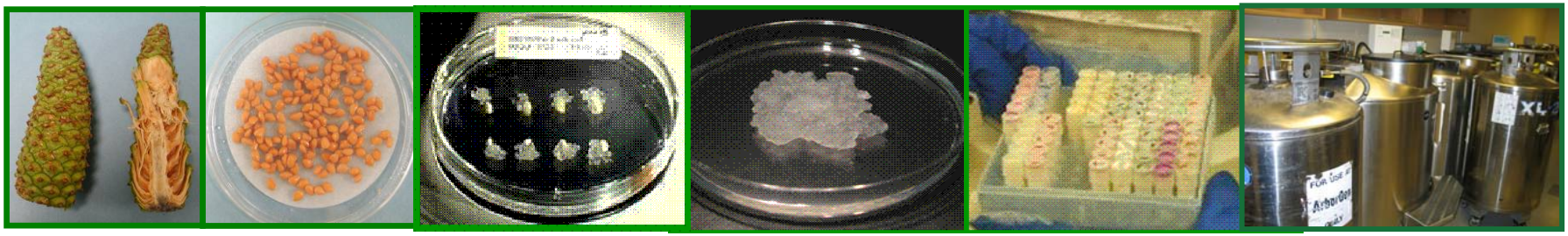




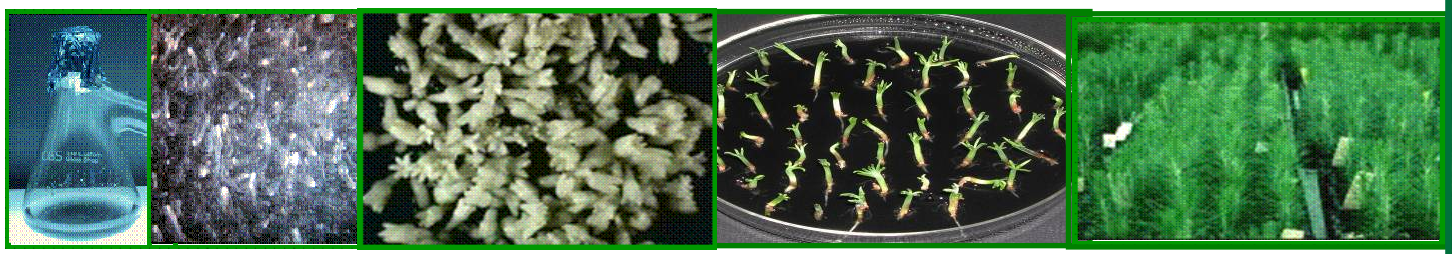
Somatic Embryogenesis (SE)-Based Varietal Technology

Improvements in Biology

Varieties Preserved

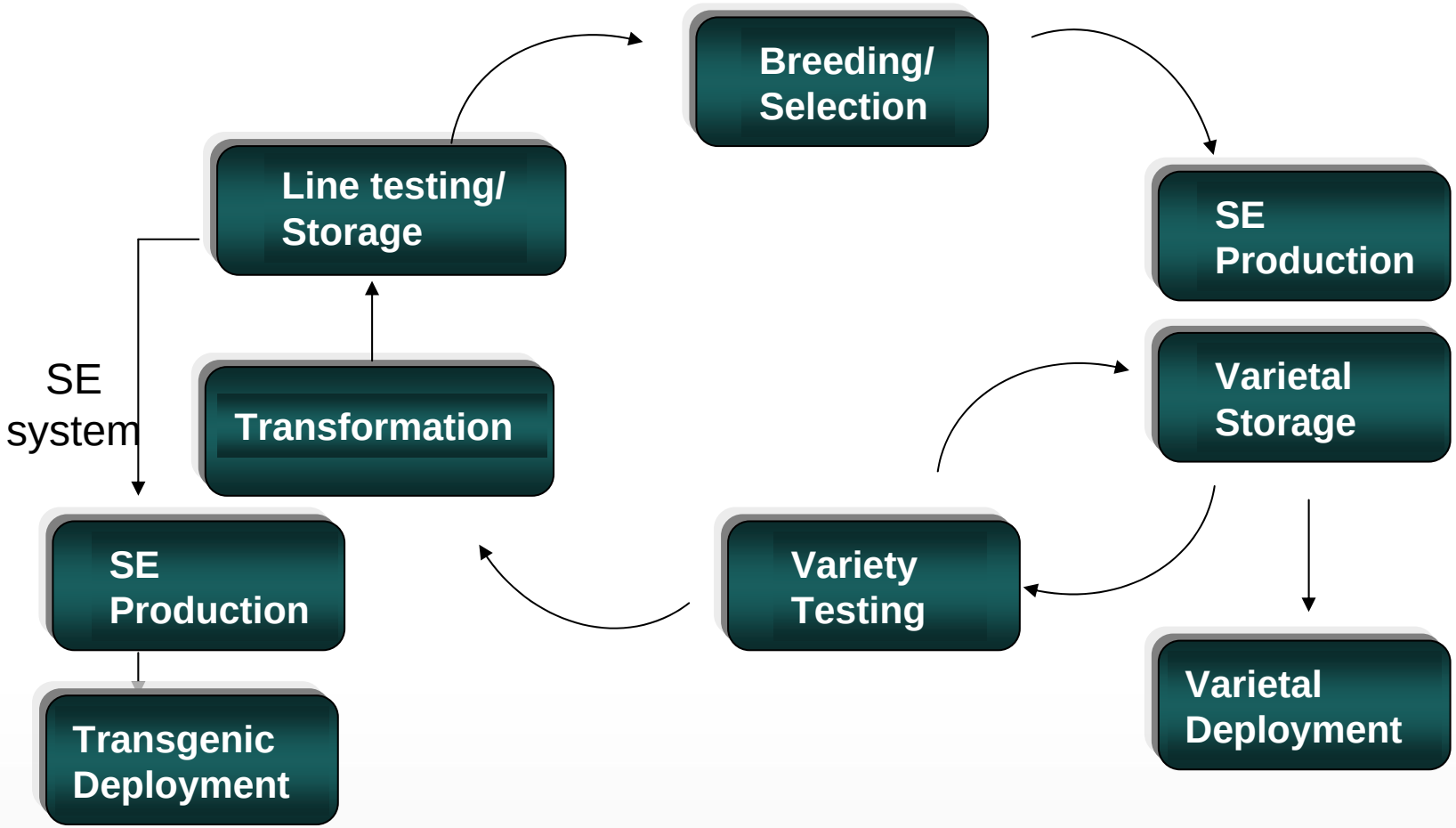


Varieties Produced, Tested and Selected

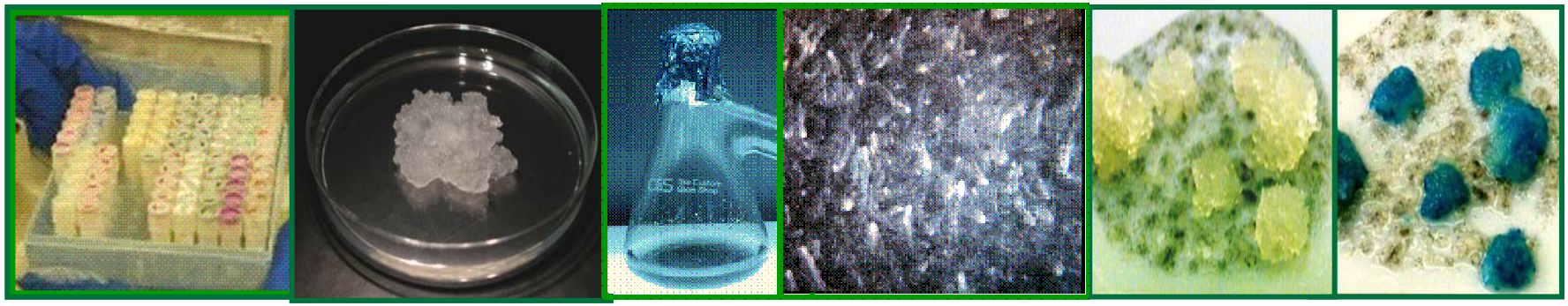


Elite Varieties Produced for Plantations

SE Varietal Technology is Essential to Loblolly Pine Product Development



Transgenic plant production is a multi-step and long process



Elite Clone

8 wks

Gene Insertion and Selection

12 wks (~1200 events from 4 constructs 3FTEs)

Bulk-up

SE Dev. Harvest

Germination Conversion

Establishment

Field planting

15 - 21 wks

19-21 wks

24-28 wks

Transgenic Plant Production and Challenges

Major Challenge = High Cost of Plants

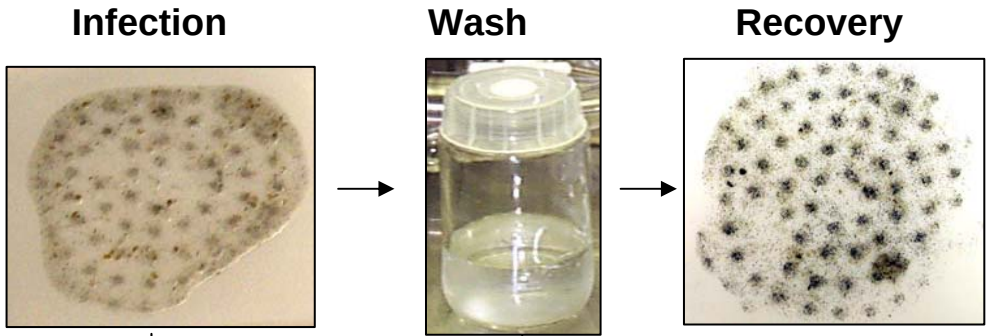
Areas that affect cost significantly

- **Efficiency**
- **Capacity**
- **SE quality**
- **Multiple labor intensive steps:**
  - **Tissue plating**
  - **Embryo harvest**
  - **Germination and sorting**

Improvements in Transformation

New liquid medium before infection improves the transformation efficiency

AG D: early cell growth medium
AG M: new cell growth medium



AG D/D
AG D/D + Tim
AG D/M
AG M/D
AG M/D + Tim
AG M/M

X

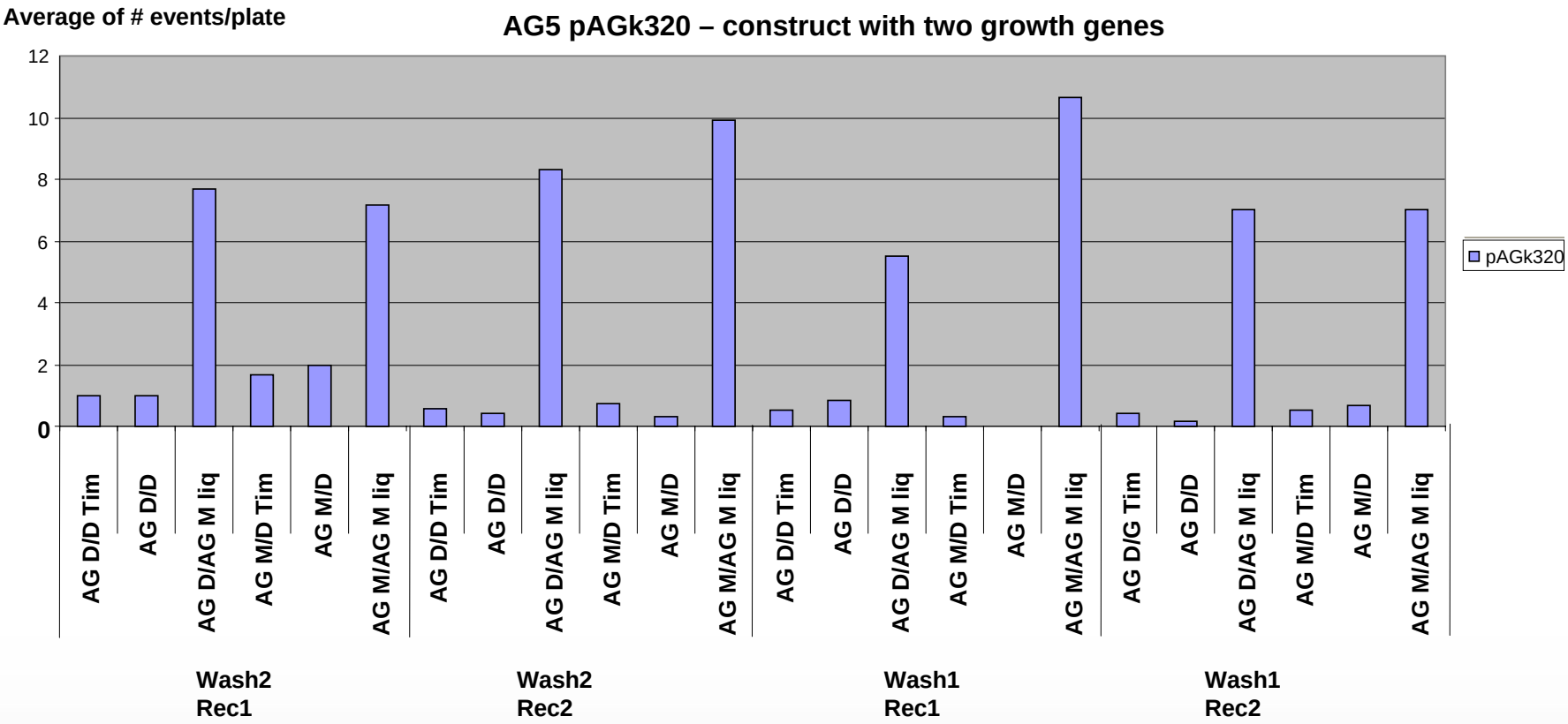
Wash1
Wash2

X

Rec1
Rec2

Improvements in Transformation

New medium before infection improves the transformation efficiency (4-10x)



Improvements in Transformation

Improved efficiency = increased capacity

Loblolly pine transgenic line production at ArborGen since 2002.

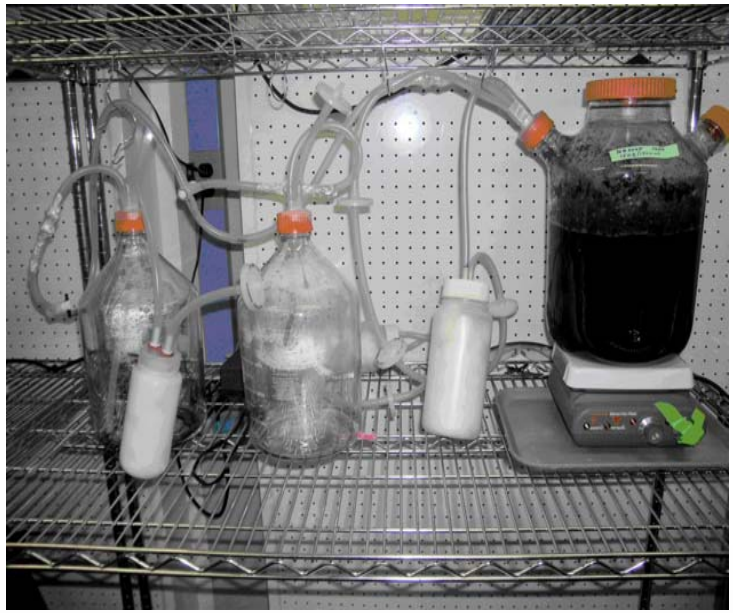
Variety	Purpose	No. of Constructs	No. of Transgenic Lines Produced*
AG10	Growth	5	783
AG6	Growth	5	151
AG3	Growth	4	1403
AG2	Growth	4	1334
AG5	Growth	4	927
AG9	Growth	4	1523
AG8	Growth	4	1035
AG7	Growth	1	72
AG1	Growth	2	172
AG4	Gene screening	157	4003
AG3	Gene screening	189	12733
AG11	Gene screening	15	395
AG12	Gene screening	7	173
AG13	Gene screening	18	1414
AG14	Gene screening	32	117
AG15	Gene screening	26	945
AG17	Gene screening	22	337
AG18	Gene screening	15	322
Total		**514**	**27839**

*Only necessary lines were taken to embryo development and plant production.

SE Scale-Up with Bioreactor

Tissue bulk-up → **Scale up with bioreactor**

Bioreactor produces large quantity of tissues for SE development



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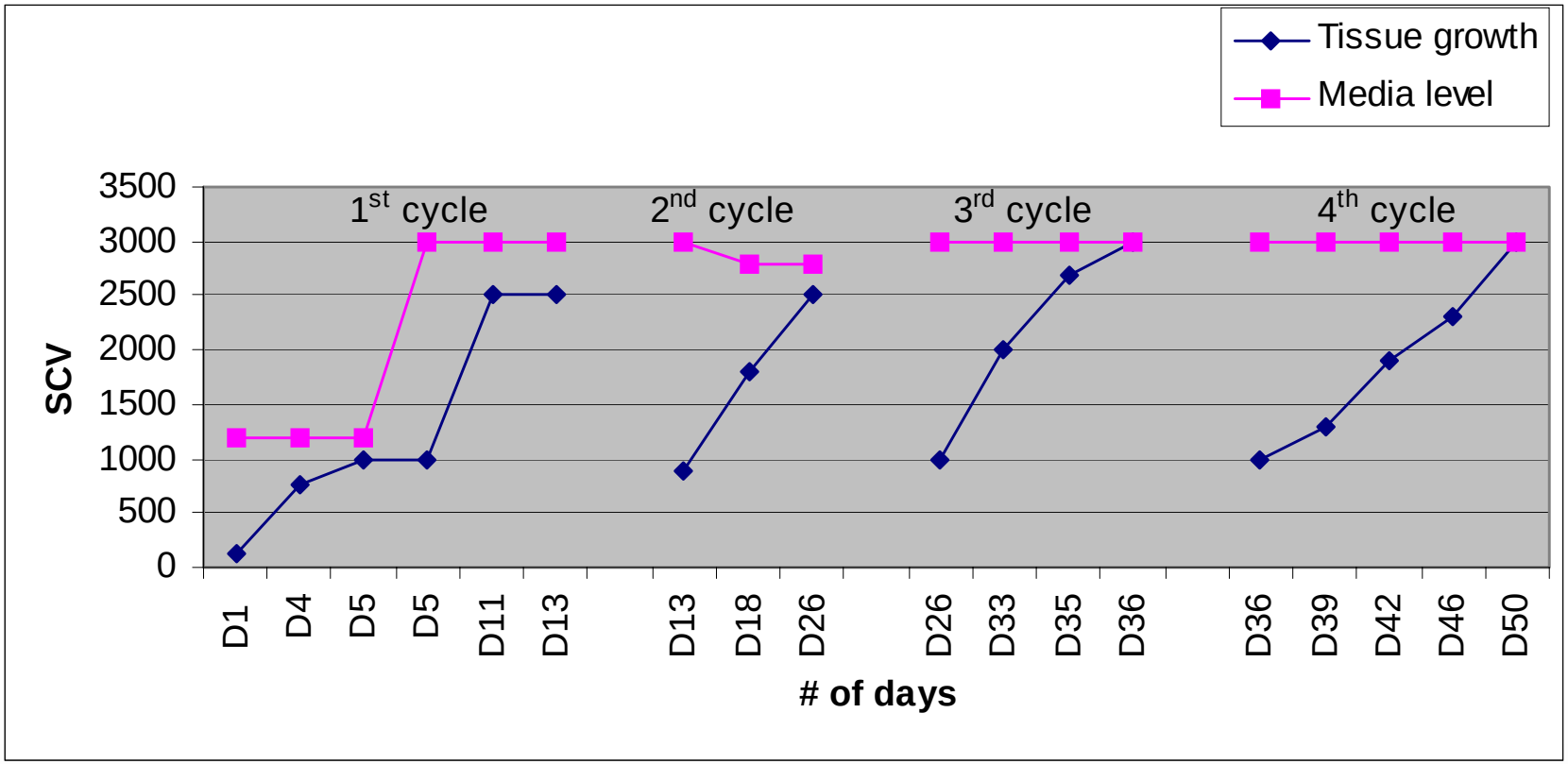
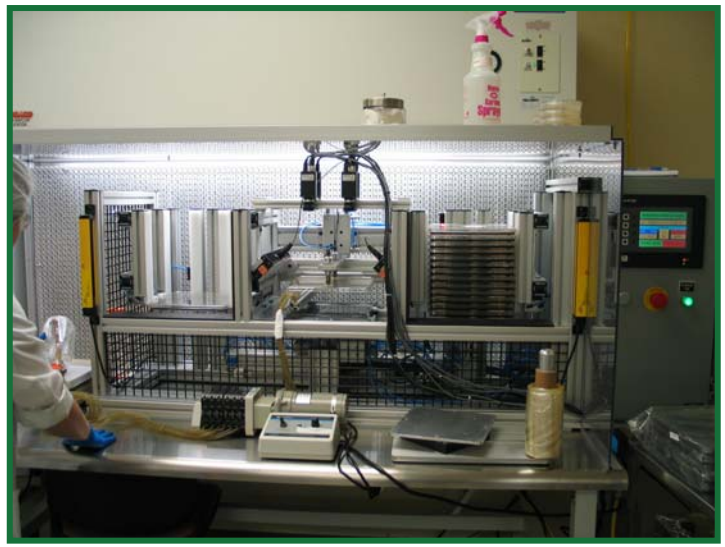


Improvements in SE Scale-Up (Automation)

SE development → **Tissue plating machine**



Machine (120 vessels/hr) vs Manual (24-55 vessels/hr): 2.2-5x increase in efficiency
(better distribution, less contamination)

Large quantity of SE cultures produce large number of embryos

Avg. 1000 SEs/vessel

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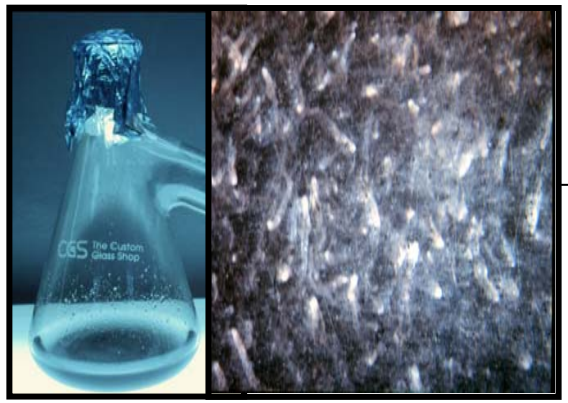
Avg. 1000 SEs/vessel

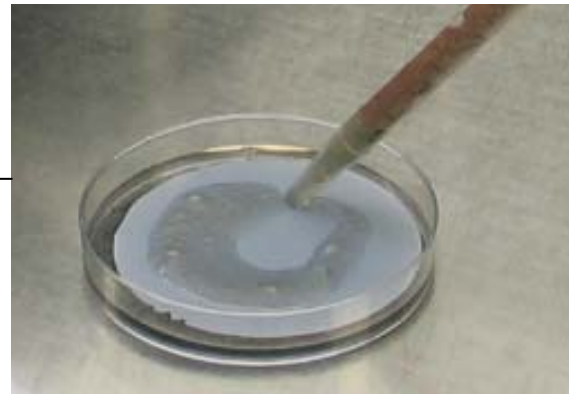
Liquid culture in SE quality improvement

Liquid culture treatment improved embryo production and quality

SE culture



Plating



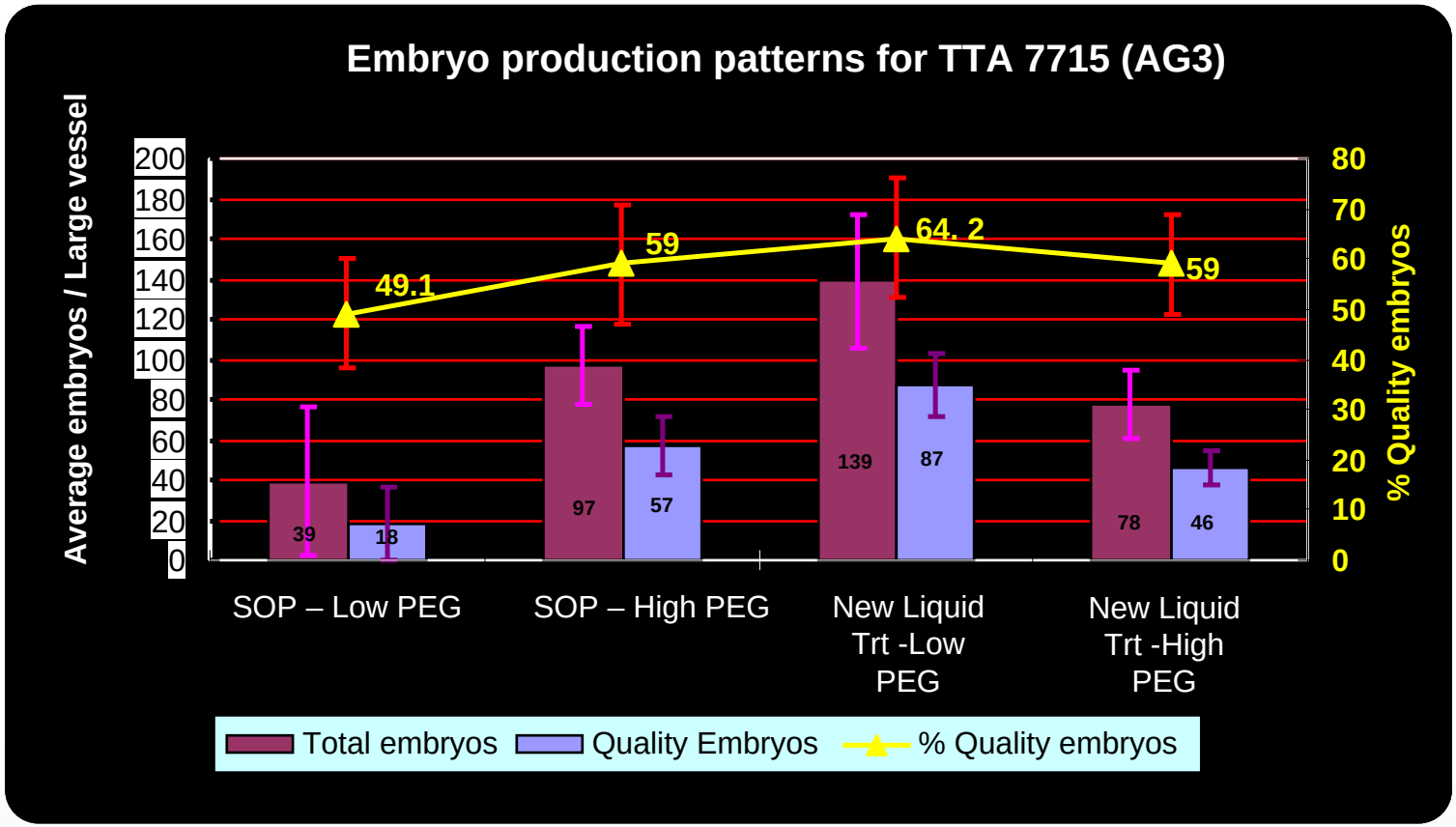
Add a liquid medium treatment for 4 weeks

Average # High Quality SEs/ml of culture of 3 Transgenic Varieties

SOP	31.0±26.9
With treatment	54.3±23.1

Improvements in SE Scale-Up (Research)

Liquid culture treatment improved embryo production and quality



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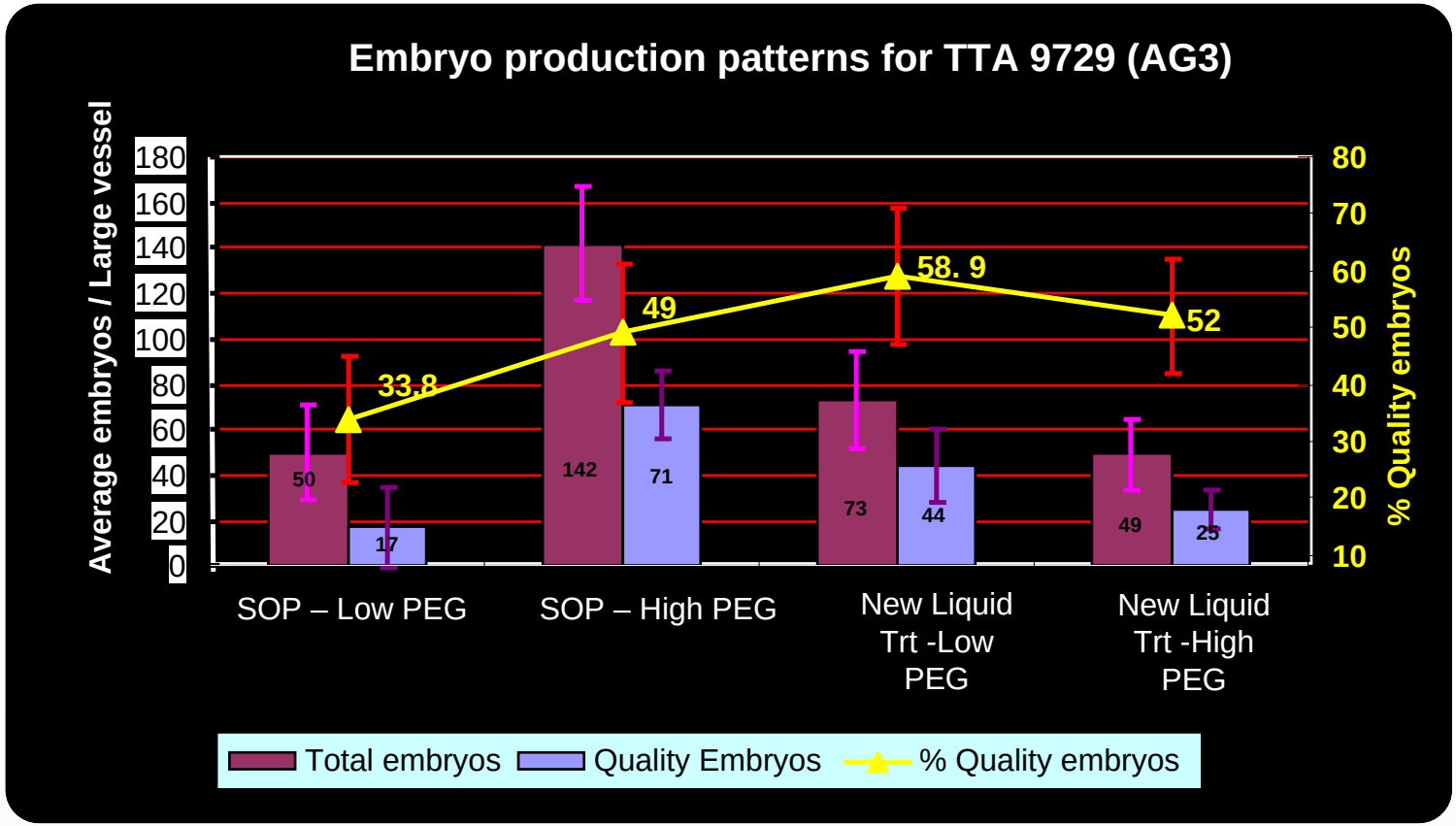

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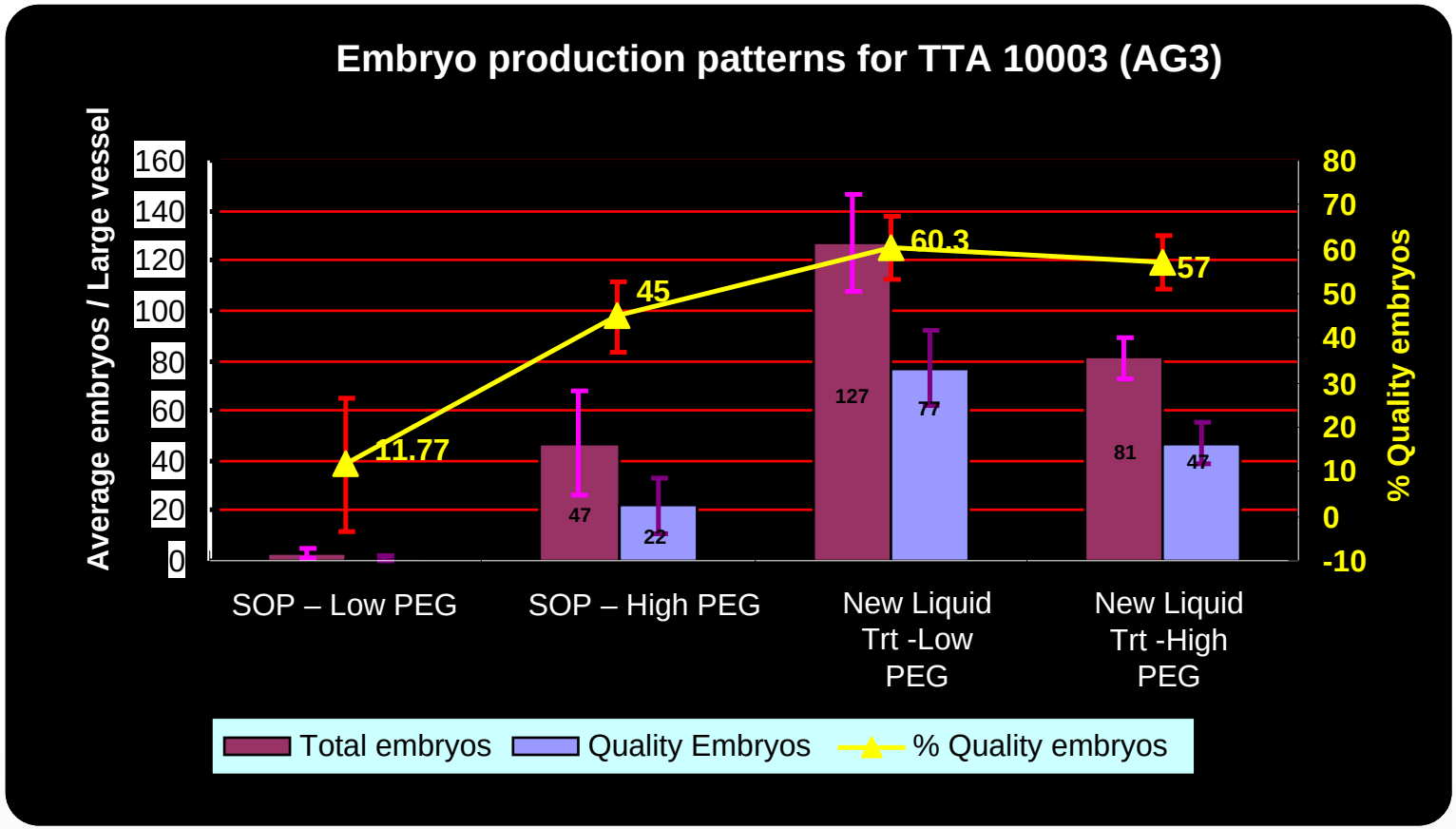
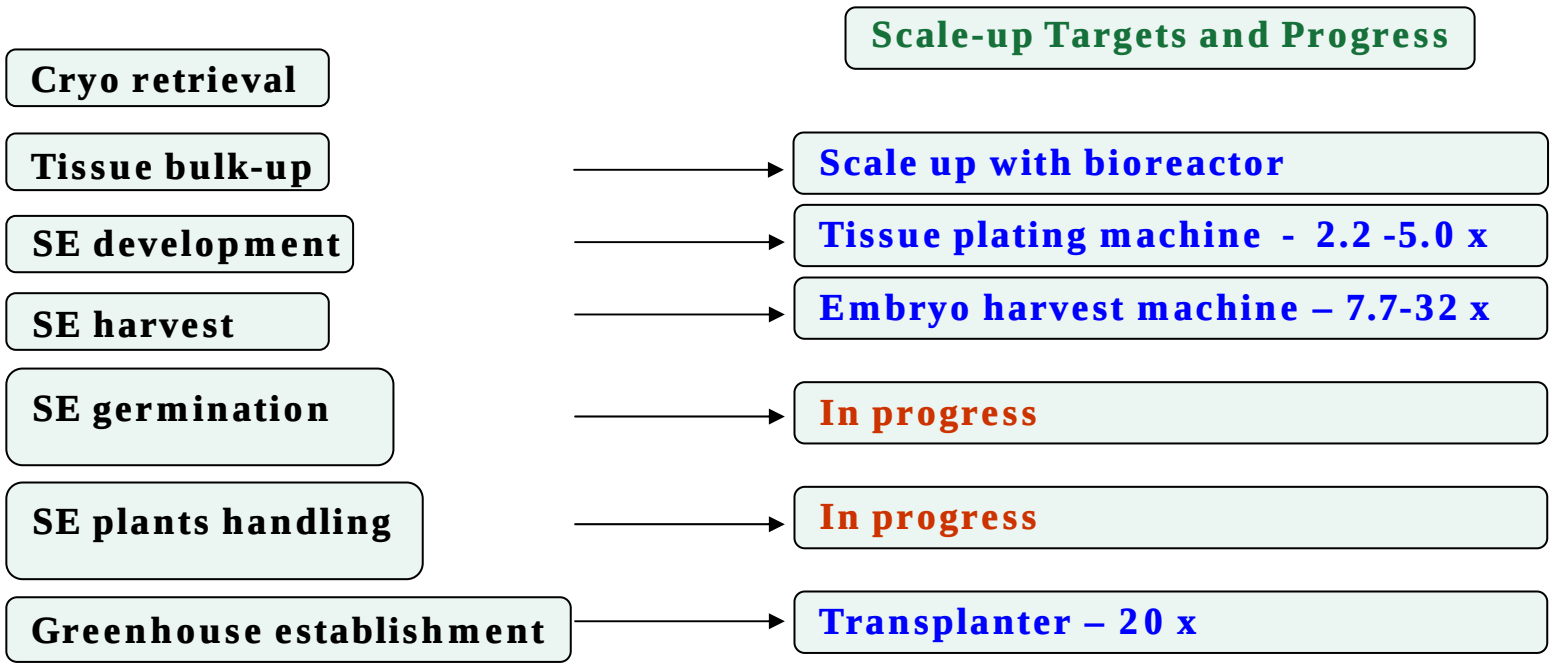


Transgenic Plant Production and Challenges



Summary

- Varietal technology and biotech offer great potential for better loblolly pine products
- High cost is the major challenge for commercial production of biotech pine
- High transformation efficiency is necessary to reduce the cost of biotech pine
- Liquid culture is a good target for major efficiency improvement
- Automation is required to increase the efficiency
- Research in SE quality and scale-up technology can lead to significant reduction in production cost

Acknowledgements

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